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(54) Title: CHLOROPHYLLASES

SEQ ID NO:19 MAAIEDSPTESS---VVTCAA-----FEIGSLPTTEIPVDPV-----ENDST
SEQ ID NO:20 MS-SSSSR-----NAFEDGKYKSNLLTLD---SSSRCKITPSSRAS
SEQ ID NO:22 MAAMVDAKPAAS---VQGTPLLA-----TATLPVETRGYISTKRITLETSSPS-----SP
SEQ ID NO:21 MAKLLLLIFGVFIFVNSQAQTFPTILEKHNSEKITOVFNKGNFQVTNNPI-----RVKRYEF
SEQ ID NO: 2 -----RVET-----SNIA-
SEQ ID NO: 4 STCSTTAA-----NVFEIGKHITVLLRAE---PGT--C---TTKSSLPV
SEQ ID NO: 6 MNLASAVRVFLSYLLVQRWMS-----EQGGGVFDQGGHSVSLTRLDEARAPPCAVRSPSSAAS
SEQ ID NO: 8 MAQRAEPILVT-----TDVFQMGNIKWQFNIDT-----SNASS
SEQ ID NO:10 MAQRAQPVLAT-----DVFQMGNIQWKQFNVDI-----SSASF
SEQ ID NO:12 MAQRAQPALAT-----TDVFQKGDHVKQFNVDI-----STASS
SEQ ID NO:14 MASSPCS-----VFVPGKYTVQLKSVE---AGTK--KARHVSSVSA
SEQ ID NO:16 -----
SEQ ID NO:18 MAAAAPAEATMKN---SAAGAEVP-----EAFVSVFQPGKLAVEAIQVDE-----NAAP
SEQ ID NO:26 MAAMA-----TTVFQAGPMEVDVKHVDK-----SMIP
1 70

(57) Abstract: This invention relates to isolated polynucleotides encoding chlorophyllases. The invention also relates to the construction of recombinant DNA fragments comprising all or a portion of the isolated chlorophyllase-encoding polynucleotides, in sense or antisense orientation, wherein expression of the recombinant DNA fragment results in production of altered levels of chlorophyllase in a transformed host cell.

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TITLE
CHLOROPHYLLASES

This application claims the benefit of U.S. Provisional Application No. 60/238,161 filed October 05, 2000.

FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding chlorophyllases in plants and seeds.

BACKGROUND OF THE INVENTION

Fruits, stems, and leaves change color in response to developmental and environmental factors. Although these tissues have very different functional, morphological, and biochemical attributes their cells undergo similar changes in ultrastructure and chloroplast composition during fruit ripening and leaf senescence. Chlorophyll breakdown is a primary biochemical event in color changes and the first step in the chlorophyll degradation pathway is catalyzed by chlorophyllase (EC 3.1.1.14). This enzyme catalyzes the hydrolysis of the phytol chain in chlorophylls or pheophytins to produce chlorophyllides or pheophorbides. Chlorophyllases of different mobilities on polyacrylamide gels have been purified from plants and algae and in some instances, like in citrus, there are two chlorophyllase bands present. It is not known what the difference in size represents.

Genes encoding chlorophyllases have been purified from Valencia oranges and *Chenopodium album*. The Valencia orange Chlase1 gene was isolated from a library prepared from mRNA extracted from the fruit peel, stems and leaves. The steady state level of Chlase1 mRNA increased with ethylene treatment (Jacob-Wilk, et al. *Plant J.* (1999) 20:653-661). Although three proteins, of different molecular weight, with chlorophyllase activity have been isolated from *Chenopodium album*, only the gene encoding one of them (CaCLH *C. album* chlorophyll-chlorophyllido hydrolase) has been identified (Tsuchiya et al. (1999) *Proc. Natl. Acad. Sci. USA* 96:15362-15367). Using sequence similarity to the *C. album* CaCLH chlorophyllase sequence two *Arabidopsis thaliana* genes have also been assigned chlorophyllase activity. Tsuchiya et al. refer to these *Arabidopsis* genes as AtCHL1 and AtCHL2, respectively. Expression of CaCLH, AtCHL1, and AtCHL2 in *E. coli* resulted in chlorophyllase activity (Tsuchiya et al., *supra*).

The CaCLH deduced amino acid sequence has between 32% and 40% sequence homology with that of AtCHL1 and AtCHL2. All three polypeptides have highly conserved regions that correspond to a conserved motif present in several

bacterial, animal, and plant lipases and hydrolases which includes a potential ATP/GTP-binding-site motif, or P-loop. Homology of the entire polypeptide of any of the chlorophyllases with other known lipases is less than 10%.

AtCHL2 was originally labeled COI1 and was isolated from an *Arabidopsis thaliana* mutant (*coi1*, for coronatine-insensitive 1) which is insensitive to methyl jasmonate (MeJA) and coronatine and produces sterile male flowers. AtCHL1 was originally labeled ATHCOR1 (for *Arabidopsis thaliana* coronatine induced) and was identified as being induced by MeJA, coronatine, and wounding (Benedetti et al. (1998) *Plant Physiol.* 116:1037-1042). Coronatine is a phytotoxin produced by some plant-pathogenic bacteria, and mimics the action of MeJA in plants. MeJA is a plant-signaling molecule involved in stress responses such as wounding and pathogen attack and is essential for pollen grain development in *Arabidopsis thaliana*.

Because of the involvement of chlorophyll degradation in plant cell senescence, identification of cDNAs encoding chlorophyllases in other plants, particularly economically important crop plants, will allow the controlled induction or postponement of senescence.

SUMMARY OF THE INVENTION

The present invention concerns isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide having chlorophyllase activity wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 26 have at least 80% sequence identity. It is preferred that the identity be at least 85%, it is preferable if the identity is at least 90%, it is more preferred that the identity be at least 95%. The present invention also relates to isolated polynucleotides comprising the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary. More specifically, the present invention concerns isolated polynucleotides encoding the polypeptide sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 26 or nucleotide sequences comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 25.

In a second embodiment, this invention relates to a vector comprising the polynucleotide of the present invention or a recombinant DNA construct comprising the polynucleotide of the present invention operably linked to at least one regulatory sequence.

In a third embodiment, the invention concerns a cell comprising the recombinant DNA construct of the present invention. The cell may be a eukaryotic cell such as a plant cell, or a prokaryotic cell such as a bacterial cell.

In a fourth embodiment, the invention relates to a method of transforming a cell by introducing into the cell a nucleic acid comprising a polynucleotide of the present invention. The invention also concerns a method for producing a plant comprising transforming a plant cell with a nucleic acid molecule comprising a polynucleotide of the present invention and regenerating a plant from the transformed plant cell. In a further embodiment, the seed from the transformed plant is included.

In a fifth embodiment the invention concerns a method for isolating a polypeptide encoded by the polynucleotide of the present invention comprising isolating the polypeptide from a cell transformed with a nucleic acid comprising said polynucleotide.

In a sixth embodiment the invention relates to an isolated chlorophyllase polypeptide comprising an amino acid sequence having a sequence identity of at least 80% when compared to an amino acid sequence having an amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 26. It is preferred that the identity be at least 85%, it is more preferred if the identity is at least 90%, it is preferable that the identity be at least 95%. The invention is related to a polypeptide of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 26.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE LISTINGS

The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application.

Figures 1A-1F show a comparison of the amino acid sequences of the grape clone vdb1c.pk002.p19:fis (SEQ ID NO:2), grape clone vrl1c.pk008.o21:fis (SEQ ID NO:4), corn clone csh3c.pk001.a9:fis (SEQ ID NO:6), soybean clone sfl1.pk0046.f8 (SEQ ID NO:8), soybean clone sfl1n1.pk002.m10:fis (SEQ ID NO:10), soybean clone sl2.pk130.f15:fis (SEQ ID NO:12), tulip clone etp1c.pk005.d16:fis (SEQ ID NO:14), wheat clone wl1.pk0012.d7:fis (SEQ ID NO:16), wheat clone wle1n.pk0058.a4:fis (SEQ ID NO:18), wheat clone wyr1c.pk005.f22:fis (SEQ ID NO:26), and the chlorophyllases from *Arabidopsis thaliana* (NCBI General Identifier Nos. 2460203 and 6729677, SEQ ID NO:19 and SEQ ID NO:20, respectively), *Chenopodium album* (NCBI General Identifier No. 6729675, SEQ ID NO:21), and *Citrus sinensis* (NCBI General Identifier No. 7328567, SEQ ID NO:22). Amino acids conserved among all the sequences are indicated by an asterisk (*) above the alignment. The lipase and P-loop motif discussed in Tsuchiya et al. (1999, *Proc. Natl. Acad. Sci. U.S.A.* 96:15362-15367) is underlined and the putative active Serine

(from the same article) is written in white and boxed in black. Dashes are used by the program to maximize the alignment. Figure 1A, amino acids 1 through 70; Figure 1B, amino acids 71 through 140; Figure 1C, amino acids 141 through 210; Figure 1D, amino acids 211-280, Figure 1E, amino acids 281 through 350; Figure 1F, amino acids 351 through 397.

Figures 2A-2B show a comparison of the amino acid sequences of the corn clone csc1c.pk006.l9: fis (SEQ ID NO:28), and the chlorophyllases from *Arabidopsis thaliana* (NCBI General Identifier Nos. 2460203 and 6729677, SEQ ID NO:19 and SEQ ID NO:20, respectively), *Chenopodium album* (NCBI General Identifier No. 6729675, SEQ ID NO:21), and *Citrus sinensis* (NCBI General Identifier No. 7328567, SEQ ID NO:22). Amino acids conserved among all the sequences are indicated by an asterisk (*) above the alignment. The lipase and P-loop motif discussed in Tsuchiya et al. (1999, *Proc. Natl. Acad. Sci. U.S.A.* 96:15362-15367) is underlined and the putative active Serine (from the same article) is written in white and boxed in black. Dashes are used by the program to maximize the alignment. Figure 2A, positions 1 through 210; Figure 2B, positions 211 through 384.

Table 1 lists the chlorophyllase polypeptides that are described herein, the plant species from which the polypeptide is derived, the designation of the cDNA clones that comprise the nucleic acid fragments encoding all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

TABLE 1
Chlorophyllases

Species	Clone Designation	SEQ ID NO:	
		(Nucleotide)	(Amino Acid)
Grape	vdb1c.pk002.p19: fis	1	2
Grape	vrl1c.pk008.o21: fis	3	4
Corn	csh3c.pk001.a9: fis	5	6
Soybean	sfl1.pk0046.f8	7	8
Soybean	sfl1n1.pk002.m10: fis	9	10
Soybean	sl2.pk130.f15: fis	11	12
Tulip	etp1c.pk005.d16: fis	13	14
Wheat	wl1.pk0012.d7: fis	15	16
Wheat	wle1n.pk0058.a4: fis	17	18
<i>A. thaliana</i> COI1	GI 2460203		19

Species	Clone Designation	SEQ ID NO:	
		(Nucleotide)	(Amino Acid)
<i>A. thaliana</i> CLH2	GI 6729677		20
<i>C. album</i> CLH	GI 6729675		21
<i>C. sinensis</i>	GI 7328567		22
Oligonucleotide Primer	Primer P1	23	
Oligonucleotide Primer	Primer P2	24	
Wheat	wyr1c.pk005.f22:fis	25	26
Corn	csc1c.pk006.l9:fis	27	28
Oligonucleotide Primer	Primer P3	29	
Oligonucleotide Primer	Primer P4	30	

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Res.* 13:3021-3030 (1985) and in the *Biochemical J.* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. The terms "polynucleotide", "polynucleotide sequence", "nucleic acid sequence", and "nucleic acid fragment"/"isolated nucleic acid fragment" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least 30 contiguous nucleotides, preferably at least 40 contiguous nucleotides, most preferably at least 60 contiguous nucleotides derived from SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, or 25 or the complement of such sequences.

The term "isolated" refers to materials, such as nucleic acid molecules and/or proteins, which are substantially free or otherwise removed from components that normally accompany or interact with the materials in a naturally occurring environment. Isolated polynucleotides may be purified from a host cell in which they naturally occur. Conventional nucleic acid purification methods known to skilled artisans may be used to obtain isolated polynucleotides. The term also

embraces recombinant polynucleotides and chemically synthesized polynucleotides.

The term "recombinant" means, for example, that a nucleic acid sequence is made by an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated nucleic acids by genetic engineering techniques.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by gene silencing through for example antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting transcript vis-à-vis the ability to mediate gene silencing or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof. The terms "substantially similar" and "corresponding substantially" are used interchangeably herein.

Substantially similar nucleic acid fragments may be selected by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the polypeptide encoded by the unmodified nucleic acid fragment in a plant or plant cell. For example, a substantially similar nucleic acid fragment representing at least 30 contiguous nucleotides, preferably at least 40 contiguous nucleotides, most preferably at least 60 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a plant or plant cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a plant or plant cell exposed to the substantially similar nucleic fragment can then be compared to the level of the polypeptide in a plant or plant cell that is not exposed to the substantially similar nucleic acid fragment.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by using nucleic acid

fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in the art.

5 Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for
10 another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity
15 of the encoded products.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) Nucleic
20 Acid Hybridisation, IRL Press, Oxford, U.K.). Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of
25 washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5%
30 SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C.

Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms
35 commonly employed by those skilled in this art. Suitable nucleic acid fragments (isolated polynucleotides of the present invention) encode polypeptides that are at least about 70% identical, preferably at least about 80% identical to the amino acid

sequences reported herein. Preferred nucleic acid fragments encode amino acid sequences that are at least about 85% identical to the amino acid sequences reported herein. More preferred nucleic acid fragments encode amino acid sequences that are at least about 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are at least about 95% identical to the amino acid sequences reported herein. Suitable nucleic acid fragments not only have the above identities but typically encode a polypeptide having at least 50 amino acids, preferably at least 100 amino acids, more preferably at least 150 amino acids, still more preferably at least 200 amino acids, and most preferably at least 250 amino acids. Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CAB/OS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also the explanation of the BLAST algorithm on the world wide web site for the National Center for Biotechnology Information at the National Library of Medicine of the National Institutes of Health). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford

specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now
5 use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

"Codon degeneracy" refers to divergence in the genetic code permitting
10 variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide
15 codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic nucleic acid fragments" can be assembled from oligonucleotide
20 building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment. "Chemically synthesized", as related to a nucleic acid fragment, means that the component nucleotides were assembled
25 *in vitro*. Manual chemical synthesis of nucleic acid fragments may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of the nucleotide sequence to reflect the codon bias of the host cell.
30 The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

"Gene" refers to a nucleic acid fragment that expresses a specific protein,
35 including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers any gene

that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but
5 arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign-gene" refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene"
10 is a gene that has been introduced into the genome by a transformation procedure.

"Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA
15 processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence
20 is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a
25 promoter. Promoters may be derived in their entirety from a native gene, or may be composed of different elements derived from different promoters found in nature, or may even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to
30 different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further
35 recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

“Translation leader sequence” refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol.* 3:225-236).

“3' non-coding sequences” refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell* 1:671-680.

“RNA transcript” refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. “Messenger RNA (mRNA)” refers to the RNA that is without introns and that can be translated into polypeptides by the cell. “cDNA” refers to DNA that is complementary to and derived from an mRNA template. The cDNA can be single-stranded or converted to double stranded form using, for example, the Klenow fragment of DNA polymerase I. “Sense-RNA” refers to an RNA transcript that includes the mRNA and so can be translated into a polypeptide by the cell. “Antisense RNA” refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific nucleotide sequence, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. “Functional RNA” refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term “operably linked” refers to the association of two or more nucleic acid fragments on a single polynucleotide so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding

sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid
5 fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein.

"Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms.

10 "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

A "protein" or "polypeptide" is a chain of amino acids arranged in a specific order determined by the coding sequence in a polynucleotide encoding the
15 polypeptide. Each protein or polypeptide has a unique function.

"Altered levels" or "altered expression" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

"Mature protein" or the term "mature" when used in describing a protein
20 refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor protein" or the term "precursor" when used in describing a protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization
25 signals.

A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. "Chloroplast transit sequence" refers to a nucleotide sequence that encodes a chloroplast transit
30 peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*)
35 may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys.* 100:1627-1632).

“Transformation” refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as “transgenic” organisms. Examples of methods of plant transformation include

5 *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or “gene gun” transformation technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference). Thus, isolated polynucleotides of the present invention can be incorporated into recombinant constructs, typically DNA constructs, capable of

10 introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels et al., *Cloning Vectors: A*

15 *Laboratory Manual*, 1985, supp. 1987; Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989; and Flevin et al., *Plant Molecular Biology Manual*, Kluwer Academic Publishers, 1990. Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable

20 marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

25 Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter “Maniatis”).

“PCR” or “polymerase chain reaction” is well known by those skilled in the art

30 as a technique used for the amplification of specific DNA segments (U.S. Patent Nos. 4,683,195 and 4,800,159).

Nucleic acid fragments encoding at least a portion of several chlorophyllases have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST

35 algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using

sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, 5 ligase chain reaction).

For example, genes encoding other chlorophyllases, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific 10 oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, an entire sequence can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In 15 addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

20 In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid 25 fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002) to 30 generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems, specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673-5677; Loh et al. 35 (1989) *Science* 243:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165). Consequently, a polynucleotide comprising a nucleotide

sequence of at least 30 (preferably at least 40, most preferably at least 60) contiguous nucleotides derived from a nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 25 and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide.

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol.* 36:1-34; Maniatis).

In another embodiment, this invention concerns viruses and host cells comprising either the recombinant DNA fragments of the invention as described herein or an isolated polynucleotide of the invention as described herein. Examples of host cells which can be used to practice the invention include, but are not limited to, yeast, bacteria, and plants.

As was noted above, the nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of chlorophyll in those cells. Manipulation of chlorophyllase activity may be useful for the controlled induction of senescence in agronomic or agricultural applications.

For example, expressing a chlorophyllase gene under the control of an inducible promoter will allow plants to mature and age earlier in nurseries, or to mature and age later for their use in florist arrangements or during shipping to retail.

Overexpression of chlorophyllase genes in oil-producing crops will result in seeds and extracted oils with reduced chlorophyll-derived pigmentation. Chlorophyllase genes may also be useful to develop male sterility in wheat. It may also be possible to use the chlorophyllase expression as a transformation selection marker where non-transformed tissue will be green and transformed tissue will be non-green.

Furthermore, chlorophyllases may be prepared in microbial systems, purified, and added to detergents where they will be used to remove grass and other stains resulting from green plant tissues.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to

a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. The chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided.

5 The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Vectors may be constructed comprising the instant isolated polynucleotides or recombinant DNA fragments. The choice of vector is dependent upon the method that will be used to transform host cells. The skilled artisan is well aware of

10 the genetic elements that must be present on the vector in order to successfully transform, select and propagate host cells containing the foreign polynucleotide. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al. (1985) *EMBO J.* 4:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics* 218:78-86),

15 and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis, among others.

For some applications it may be useful to direct the instant polypeptides to

20 different cellular compartments, or to facilitate their secretion from the cell. It is thus envisioned that the recombinant DNA fragment described above may be further supplemented by directing the coding sequence to encode the instant polypeptides with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell* 56:247-253), signal sequences or sequences encoding

25 endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.* 100:1627-1632) with or without removing targeting sequences that are already present. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of use may be discovered in the future.

30 It may also be desirable to reduce or eliminate expression of genes encoding the instant polypeptides in plants for some applications. In order to accomplish this, a chimeric gene designed for co-suppression of the instant polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences. Alternatively, a chimeric gene designed to express antisense

35 RNA for all or part of the instant nucleic acid fragment can be constructed by linking the gene or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into

plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

5 Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U.S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or cosuppression construct would act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of gene activity 10 these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a breeding perspective. In addition, the ability to restrict the expression of a specific phenotype to the reproductive tissues of the plant by the use of tissue specific promoters may confer agronomic advantages relative to conventional mutations which may have an effect 15 in all tissues in which a mutant gene is ordinarily expressed.

The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different chimeric genes 20 utilizing different regulatory elements known to the skilled artisan. Once transgenic plants are obtained by one of the methods described above, it will be necessary to screen individual transgenics for those that most effectively display the desired phenotype. Accordingly, the skilled artisan will develop methods for screening large numbers of transformants. The nature of these screens will generally be chosen on 25 practical grounds. For example, one can screen by looking for changes in gene expression by using antibodies specific for the protein encoded by the gene being suppressed, or one could establish assays that specifically measure enzyme activity. A preferred method will be one which allows large numbers of samples to be processed rapidly, since it will be expected that a large number of transformants 30 will be negative for the desired phenotype.

The instant chlorophyllase polypeptides (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of 35 the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that

direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a recombinant DNA fragment for production of the instant polypeptides. This recombinant DNA fragment could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded chlorophyllase. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 6).

All or a substantial portion of the polynucleotides of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and used as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics* 1:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet.* 32:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter* 4:37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

Nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favor use of

large clones (several to several hundred KB; see Laan et al. (1995) *Genome Res.* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

5 A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med.* 11:95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics* 16:325-332), allele-specific ligation (Landegren et al. (1988) *Science* 241:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res.* 10 18:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nat. Genet.* 7:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In 15 methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA 20 clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger and Benzer (1989) *Proc. Natl. Acad. Sci USA* 86:9402-9406; Koes et al. (1995) *Proc. Natl. Acad. Sci USA* 92:8149-8153; Bensen et al. (1995) *Plant Cell* 7:75-84). The latter approach may be accomplished in two ways. First, short 25 segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of 30 the mutation tag element in or near the plant gene encoding the instant polypeptides. Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic 35 adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptides can be identified and obtained. This mutant plant

can then be used to determine or confirm the natural function of the instant polypeptides disclosed herein.

EXAMPLES

The present invention is further defined in the following Examples, in which parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosure of each reference set forth herein is incorporated herein by reference in its entirety.

EXAMPLE 1

Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various grape, corn, soybean, tulip, and wheat tissues were prepared. The characteristics of the libraries are described below.

TABLE 2

cDNA Libraries from Grape, Corn, Soybean, Tulip, and Wheat

Library	Tissue	Clone
vdb1c	Grape Developing Bud	vdb1c.pk002.p19:fis
vr1c	Grape Resistant Leaves	vr1c.pk008.o21:fis
csh3c	Corn Shoots and Roots Sprayed With Herbicide*	csh3c.pk001.a9:fis
sfl1	Soybean Immature Flower	sfl1.pk0046.f8
sfl1n1	Soybean Immature Flower**	sfl1n1.pk002.m10:fis
sl2	Soybean Two-Week-Old Developing Seedlings Treated With 2.5 ppm chlorimuron	sl2.pk130.f15:fis
etp1c	Tulip Pistil Developed to 3/4 of its Mature Size	etp1c.pk005.d16:fis
wl1	Wheat Leaf From 7 Day Old Seedling	wl1.pk0012.d7:fis
wle1n	Wheat Leaf From 7 Day Old Etiolated Seedling**	wle1n.pk0058.a4:fis
wyr1c	Wheat yellow rust infested tissue	wyr1c.pk005.f22:fis
csc1c	Corn 20-Day Seedling (Germination Cold Stress). The Seedling Appeared Purple.	csc1c.pk006.l9:fis

* Application of *N*-(3,6-dihydro-2*H*-pyran-4-yl)-4-(3,5-dimethyl-4-isoxazolyl)-4,5-dihydro-*N*-(1-methylethyl)-5-oxo-1*H*-tetrazole-1-carboxamide; synthesis and methods of using this compound are described in WO98/35961, incorporated herein by reference.

- 5 **These libraries were normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the
10 cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by
15 transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase
20 chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science* 252:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent
25 sequencer.

Full-insert sequence (FIS) data is generated utilizing a modified transposition protocol. Clones identified for FIS are recovered from archived glycerol stocks as single colonies, and plasmid DNAs are isolated via alkaline lysis. Isolated DNA
30 templates are reacted with vector primed M13 forward and reverse oligonucleotides in a PCR-based sequencing reaction and loaded onto automated sequencers. Confirmation of clone identification is performed by sequence alignment to the original EST sequence from which the FIS request is made.

Confirmed templates are transposed via the Primer Island transposition kit (PE Applied Biosystems, Foster City, CA) which is based upon the *Saccharomyces cerevisiae* Ty1 transposable element (Devine and Boeke (1994) *Nucleic Acids Res.*
35 22:3765-3772). The *in vitro* transposition system places unique binding sites randomly throughout a population of large DNA molecules. The transposed DNA is then used to transform DH10B electro-competent cells (Gibco BRL/Life Technologies, Rockville, MD) via electroporation. The transposable element

contains an additional selectable marker (named DHFR; Fling and Richards (1983) *Nucleic Acids Res.* 11:5147-5158), allowing for dual selection on agar plates of only those subclones containing the integrated transposon. Multiple subclones are randomly selected from each transposition reaction, plasmid DNAs are prepared via alkaline lysis, and templates are sequenced (ABI Prism dye-terminator ReadyReaction mix) outward from the transposition event site, utilizing unique primers specific to the binding sites within the transposon.

Sequence data is collected (ABI Prism Collections) and assembled using Phred/Phrap (P. Green, University of Washington, Seattle). Phrep/Phrap is a public domain software program which re-reads the ABI sequence data, re-calls the bases, assigns quality values, and writes the base calls and quality values into editable output files. The Phrap sequence assembly program uses these quality values to increase the accuracy of the assembled sequence contigs. Assemblies are viewed by the Consed sequence editor (D. Gordon, University of Washington, Seattle).

In some of the clones the cDNA fragment corresponds to a portion of the 3'-terminus of the gene and does not cover the entire open reading frame. In order to obtain the upstream information one of two different protocols are used. The first of these methods results in the production of a fragment of DNA containing a portion of the desired gene sequence while the second method results in the production of a fragment containing the entire open reading frame. Both of these methods use two rounds of PCR amplification to obtain fragments from one or more libraries. The libraries some times are chosen based on previous knowledge that the specific gene should be found in a certain tissue and some times are randomly-chosen. Reactions to obtain the same gene may be performed on several libraries in parallel or on a pool of libraries. Library pools are normally prepared using from 3 to 5 different libraries and normalized to a uniform dilution. In the first round of amplification both methods use a vector-specific (forward) primer corresponding to a portion of the vector located at the 5'-terminus of the clone coupled with a gene-specific (reverse) primer. The first method uses a sequence that is complementary to a portion of the already known gene sequence while the second method uses a gene-specific primer complementary to a portion of the 3'-untranslated region (also referred to as UTR). In the second round of amplification a nested set of primers is used for both methods. The resulting DNA fragment is ligated into a pBluescript vector using a commercial kit and following the manufacturer's protocol. This kit is selected from many available from several vendors including Invitrogen (Carlsbad, CA), Promega Biotech (Madison, WI), and

Gibco-BRL (Gaithersburg, MD). The plasmid DNA is isolated by alkaline lysis method and submitted for sequencing and assembly using Phred/Phrap, as above.

EXAMPLE 2

Identification of cDNA Clones

5 cDNA clones encoding chlorophyllases were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also the explanation of the BLAST algorithm on the world wide web site for the National Center for Biotechnology Information at the National Library of Medicine of
10 the National Institutes of Health) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were
15 analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein
20 sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet.* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the
25 likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

ESTs submitted for analysis are compared to the genbank database as described above. ESTs that contain sequences more 5- or 3-prime can be found by using the BLASTn algorithm (Altschul et al (1997) *Nucleic Acids Res.* 25:3389-3402)
30 against the Du Pont proprietary database comparing nucleotide sequences that share common or overlapping regions of sequence homology. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences can be assembled into a single contiguous nucleotide sequence, thus extending the original fragment in either the 5 or 3 prime direction. Once the most
35 5-prime EST is identified, its complete sequence can be determined by Full Insert Sequencing as described in Example 1. Homologous genes belonging to different species can be found by comparing the amino acid sequence of a known gene (from either a proprietary source or a public database) against an EST database

using the tBLASTn algorithm. The tBLASTn algorithm searches an amino acid query against a nucleotide database that is translated in all 6 reading frames. This search allows for differences in nucleotide codon usage between different species, and for codon degeneracy.

EXAMPLE 3

Characterization of cDNA Clones Encoding Chlorophyllases

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to chlorophyllases from *Arabidopsis thaliana* (NCBI General Identifier No. 2460203 and 6729677), *Chenopodium album* (NCBI General Identifier No. 6729675), and *Citrus sinensis* (NCBI General Identifier No. 7328567). Shown in Table 3 are the BLAST results for the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS") or for the sequences of FISs encoding entire chlorophyllases ("CGS"):

TABLE 3

BLAST Results for Sequences Encoding Polypeptides
Homologous to Chlorophyllase

Clone	Status	BLAST pLog Score			
		2460203	6729677	6729675	7328567
vdb1c.pk002.p19:fis	FIS	78.22	75.30	64.52	74.70
vrl1c.pk008.o21:fis	FIS	67.15	113.00	49.52	61.52
csh3c.pk001.a9:fis	CGS	54.30	78.40	45.70	53.70
sfl1.pk0046.f8	CGS	78.00	68.40	59.00	na
sfl1n1.pk002.m10:fis	CGS	80.04	66.40	62.10	66.30
sl2.pk130.f15:fis	CGS	76.30	69.30	60.10	66.70
etp1c.pk005.d16:fis	CGS	65.52	87.40	52.10	62.40
wl1.pk0012.d7:fis	FIS	38.30	35.40	35.00	31.00
wle1n.pk0058.a4:fis	CGS	76.00	68.00	55.40	61.04
wyr1c.pk005.f22:fis	CGS	54.40	56.10	51.10	49.00

The nucleotide sequence from the entire cDNA insert in clone vdb1c.pk002.p19:fis is shown in SEQ ID NO:1. Nucleotides 1 through 855 from SEQ ID NO:1 encode the amino acid sequence of SEQ ID NO:2. The nucleotide sequence from the entire cDNA insert in clone vrl1c.pk008.o21:fis is shown in SEQ ID NO:3. Nucleotides 1 through 933 from SEQ ID NO:3 encode the amino acid sequence of SEQ ID NO:4. The nucleotide sequence from the entire cDNA insert in clone csh3c.pk001.a9:fis is shown in SEQ ID NO:5 and it encodes an entire corn chlorophyllase. Nucleotides 71 through 1106 from SEQ ID NO:5 encode the amino

acid sequence of SEQ ID NO:6 with nucleotides 107-109 corresponding to a stop codon. The nucleotide sequence from the entire cDNA insert in clone sfl1.pk0046.f8 is shown in SEQ ID NO:7 and it encodes an entire soybean chlorophyllase. Nucleotides 12 through 953 from SEQ ID NO:7 encode the amino acid sequence of SEQ ID NO:8 with nucleotides 954-956 corresponding to a stop codon. The nucleotide sequence from the entire cDNA insert in clone sfl1n1.pk002.m10.fis is shown in SEQ ID NO:9 and it encodes an entire soybean chlorophyllase. Nucleotides 26 through 964 from SEQ ID NO:9 encode the amino acid sequence of SEQ ID NO:10 with nucleotides 965-967 corresponding to the stop codon. The nucleotide sequence from the entire cDNA insert in clone sl2.pk130.f15.fis is shown in SEQ ID NO:11 and it encodes an entire soybean chlorophyllase. Nucleotides 42 through 989 from SEQ ID NO:11 encode the amino acid sequence of SEQ ID NO:12 with nucleotides 990-992 corresponding to the stop codon. The nucleotide sequence from the entire cDNA insert in clone etp1c.pk005.d16.fis is shown in SEQ ID NO:13 and it encodes an entire tulip chlorophyllase. Nucleotides 97 through 1104 from SEQ ID NO:13 encode the amino acid sequence of SEQ ID NO:14 with nucleotides 1105-1107 corresponding to the stop codon. The nucleotide sequence from the entire cDNA insert in clone wl1.pk0012.d7.fis is shown in SEQ ID NO:15. Nucleotides 1 through 615 from SEQ ID NO:15 encode the amino acid sequence of SEQ ID NO:16 with nucleotides 616-618 corresponding to the stop codon. The nucleotide sequence from the entire cDNA insert in clone wle1n.pk0058.a4.fis is shown in SEQ ID NO:17 and it encodes an entire wheat chlorophyllase. Nucleotides 47 through 1003 from SEQ ID NO:17 encode the amino acid sequence of SEQ ID NO:18 with nucleotides 1004-1006 corresponding to the stop codon. The nucleotide sequence from the entire cDNA insert in clone wyr1c.pk005.f22.fis is shown in SEQ ID NO:25 and it encodes an entire wheat chlorophyllase. Nucleotides 57 through 1025 from SEQ ID NO:25 encode the amino acid sequence of SEQ ID NO:26 with nucleotides 1026-1028 corresponding to the stop codon.

Nucleotides 286 through 669 from soybean clone sfl1.pk0046.f8 are 98 % identical to nucleotides 6 through 390 of an EST found in the NCBI database having gi No. 6135059. Nucleotides 24 through 299 from soybean clone sl2.pk130.f15.fis are 100 % identical to nucleotides 1 through 276 of an EST found in the NCBI database having gi No. 7284173. Nucleotides 865 through 1101 from soybean clone sl2.pk130.f15.fis are 100 % identical to nucleotides 18 through 254 of an EST found in the NCBI database having gi No. 6914327. The three EST sequences

mentioned above and found in the NCBI database are identified as having similarities with the coronatine induced protein 1.

Figures 1A-1F present an alignment of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, and 26 with the AtCHL1 sequence (SEQ ID NO:19), CaCHL sequence (SEQ ID NO:21), AtCHL2 sequence (SEQ ID NO:20), and the *Citrus sinensis* chlorophyllase sequence (SEQ ID NO:22). The lipase and P-loop motif discussed in Tsuchiya et al. (1999, *Proc. Natl. Acad. Sci. U.S.A.* 96:15362-15367) is underlined and the putative active Serine is written in white and boxed in black. As can be seen in these figures, the amino acid sequences of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, and 26 have a conserved motif comprising (Leu/Ile)-(Ser/Val/Ala)-Gly-His-Ser-(Arg/Lys)-Gly-Gly-(Lys/Gln)-(Thr/Val/Asp)-(Ala/Val)-Phe-(Ala/Ser)-(Leu/Val)-Ala-Leu which includes the putative active Serine.

The data in Table 4 presents the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, and 26, with the AtCHL1 sequence (NCBI General Identifier No. 2460203, SEQ ID NO:19), the CaCHL sequence (NCBI General Identifier No. 6729675, SEQ ID NO:21), the AtCHL2 sequence (NCBI General Identifier No. 6729677, SEQ ID NO:20), and the *Citrus sinensis* chlorophyllase sequence (NCBI General Identifier No. 7328567, SEQ ID NO:22).

TABLE 4

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Chlorophyllases

Clone	SEQ ID NO.	Percent Identity to			
		2460203	6729677	7328567	6729675
vdb1c.pk002.p19:fis	2	49.1	48.4	45.6	43.5
vrl1c.pk008.o21:fis	4	40.5	58.8	36.0	32.5
csh3c.pk001.a9:fis	6	32.7	44.0	32.2	29.5
sfl1.pk0046.f8	8	42.5	38.4	40.0	36.8
sfl1n1.pk002.m10:fis	10	44.7	39.6	39.9	38.0
sl2.pk130.f15:fis	12	43.4	398.9	40.8	36.7
etp1c.pk005.d16:fis	14	39.2	47.5	35.0	33.9
wl1.pk0012.d7:fis	16	36.6	36.6	33.7	38.0
wle1n.pk0058.a4:fis	18	42.0	38.4	37.3	33.9
wyr1c.pk005.f22:fis	26	31.1	34.3	31.0	32.8

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed

using the Clustal method of alignment (Higgins and Sharp (1989) CAB/OS. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS
5 SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode one entire corn, three entire soybean, one entire tulip, one entire wheat, and a substantial portion of two grape and one wheat chlorophyllases. These are the first corn, soybean, tulip, wheat, and grape sequences known to Applicant to encode
10 chlorophyllases.

EXAMPLE 4

Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptides in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to
15 the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted
20 into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the
25 Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb Sall-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-Sall fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert
30 DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue®; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase® DNA Sequencing Kit; U.S.
35 Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al. (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos.

10 The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

20

The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 μ m in diameter) are coated with DNA using the following technique. Ten μ g of plasmid DNAs are added to 50 μ L of a suspension of gold particles (60 mg per mL). Calcium chloride (50 μ L of a 2.5 M solution) and spermidine free base (20 μ L of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 μ L of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 μ L of ethanol. An aliquot (5 μ L) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

30

35

For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a

circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture
5 membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains bialophos (5 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing bialophos. After 6 weeks, areas of
10 about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the bialophos-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After
15 two weeks the tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology* 8:833-839).

EXAMPLE 5

Expression of Chimeric Genes in Dicot Cells

A seed-specific expression cassette composed of the promoter and
20 transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides
25 downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites NcoI (which includes the ATG translation initiation codon), SmaI, KpnI and XbaI. The entire cassette is flanked by HindIII sites.

The cDNA fragment of this gene may be generated by polymerase chain
30 reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

35 Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature

seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) 327:70-73, U.S. Patent No. 4,945,050). A DuPont Biolistic® PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al. (1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the instant polypeptides and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 µL of a 60 mg/mL 1 µm gold particle suspension is added (in order): 5 µL DNA (1 µg/µL), 20 µL spermidine (0.1 M), and 50 µL CaCl₂ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 µL 70% ethanol and resuspended in 40 µL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five µL of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three

times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

EXAMPLE 6

Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene* 56:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoRI and HindIII sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoRI and HindIII sites was inserted at the BamHI site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the NdeI site at the position of translation initiation was converted to an NcoI site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% low melting agarose gel. Buffer and agarose contain 10 µg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies, Madison, WI) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 µL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs (NEB), Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized

with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 µg/mL ampicillin. Transformants containing the gene encoding the instant polypeptides are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol.* 189:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 µL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One µg of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

EXAMPLE 7

Determination of Chlorophyllase Activity in Clone wle1n.pk0058.a4

To establish its functional identity, the cDNA insert in clone wle1n.pk0058.a4 was expressed in bacteria and assayed for chlorophyllase activity. Initially, the entire cDNA insert in clone wle1n.pk0058.a4 was amplified by PCR using *Pfu* polymerase (Stratagene) and the oligonucleotide primers P1 (sense, SEQ ID NO:23) and P2 (antisense, SEQ ID NO:24):

P1: 5'-TTTCATATGGCAGCAGCAGCACCCG-3' (SEQ ID NO:23)

P2: 5'-TTTCTCGAGCTGCAATGGTACTTCTGCTC-3'. (SEQ ID NO:24)

The amplified fragment was cloned into pCR-Script Amp SK(+) (Stratagene) according to the manufacturer's protocol. The resulting plasmid was digested with *Nde*I and *Xho*I and the fragment corresponding to the cDNA insert from clone wle1n.pk0058.a4 was purified and inserted into the corresponding sites of the *E. coli* expression vector pET24a (Novagen). The resulting plasmid containing the cDNA insert from clone wle1n.pk0058.a4 in the pET24a vector was named pWhtCHL.

Recombinant protein was produced by expression of pWhtCHL in the *E. coli* strain BL21(DE3). A 35 mL culture of cells harboring pWhtCHL was grown in LB media to an absorbance at 600 nm of 0.5 and induction of the recombinant protein was accomplished by addition of IPTG to a final concentration of 0.4 mM. The cells
5 were grown for an additional 4 hours and harvested by centrifugation. To obtain a crude protein extract, cells were resuspended in 3 mL of lysis buffer (50 mM Tris-HCl (pH7.5), 38 μ M octylglucoside, and 1 mM PMSF [phenylmethanesulfonyl fluoride]). The cells were then lysed using a French pressure cell. The protein content in the resulting crude extract was determined according to the method of
10 Bradford (Bradford, M.M. (1976) *Anal. Biochem.* 72: 248-254). Separation of this extract by SDS-polyacrylamide gel electrophoresis and staining with coomassie revealed the presence of a strongly-expressed polypeptide of approximately 35 kDa that was absent from extracts of induced cells harboring the pET24a vector without insert. This 35 kDa induced protein corresponds to the size expected for a
15 polypeptide derived from the cDNA insert in clone wle1n.pk0058.a4.

The chlorophyllase activity assay was modified from that described by Tsuchiya *et al.* (1997) *Plant Cell Physiol.* 40:104-108). The protein concentration varied depending on whether the assay was done on the vector control or the
20 samples expressing the chlorophyllase. Considerably less protein from the chlorophyllase samples was used because the activity was much, much greater than that in the vector control. Extracts from *E. coli* expressing the wheat chlorophyllase contained 0.14 μ g of protein while extracts from *E. coli* containing the vector alone contained 10.5 μ g of protein. Reactions were conducted in a total
25 volume of 0.5 mL, consisting of 0.4 mL of crude protein prepared above diluted in the lysis buffer and 0.1 mL of a crude chlorophyll extract dissolved in acetone. The concentration of chlorophyll *a* in the extract was 0.88 μ M. The assay was conducted for 5 min with rapid shaking (285 rpm) in order to insure that the acetone
30 phase was dispersed in the reaction buffer. The reaction was stopped with the addition of 1.5 mL of acetone:hexane (1:2, v/v) and 0.5 mL of 2M Tris (pH9.0). After shaking the reaction tubes to homogenization, the organic and aqueous phases were partitioned by centrifugating the tubes at 2500x *g* for 10 minutes. The aqueous phase containing the chlorophyllide *a* reaction product was recovered, and its absorbance was measured at 667 nm. The amount of chlorophyllide *a* generated in the assay was determined using the extinction coefficient 76.79 mM⁻¹cm⁻¹.

35 From assays conducted as described above, chlorophyllase activity was found to be nearly 900-fold greater in crude extracts of *E. coli* expressing pWhtCHL than in extracts from *E. coli* expressing the pET24a vector without insert. These results are

thus consistent with the functional identification of the cDNA insert in clone wle1n.pk0058.a4 as encoding a chlorophyllase.

EXAMPLE 8

Identification of a Corn Clone Encoding a Different Chlorophyllase Isozyme

The BLASTX search using the EST sequences from clone csc1c.pk006.l9:fis revealed similarity of the polypeptides encoded by the cDNAs to chlorophyllases from *Arabidopsis thaliana* (NCBI General Identifier No. 2460203 and 6729677), *Chenopodium album* (NCBI General Identifier No. 6729675), and *Citrus sinensis* (NCBI General Identifier No. 7328567). Shown in Table 5 are the BLAST results for the sequences of the entire cDNA insert comprising the indicated cDNA clone and encoding entire chlorophyllase ("CGS"):

TABLE 5

BLAST Results for Sequences Encoding Polypeptides Homologous to Chlorophyllase

Clone	Status	BLAST pLog Score			
		2460203	6729677	6729675	7328567
csc1c.pk006.l9:fis	CGS	55.15	54.30	47.15	45.52

The nucleotide sequence from the entire cDNA insert in clone csc1c.pk006.l9:fis is shown in SEQ ID NO:27 and it encodes an entire corn chlorophyllase. Nucleotides 72 through 1069 from SEQ ID NO:27 encode the amino acid sequence of SEQ ID NO:28 with nucleotides 1070-1072 corresponding to the stop codon. When this corn chlorophyllase is compared with other chlorophyllases it shows an amino acid insertion close to the N-terminus of the protein.

Figures 2A-2B present an alignment of the amino acid sequences set forth in SEQ ID NO:28 with the AtCHL1 sequence (SEQ ID NO:19), CaCHL sequence (SEQ ID NO:21), AtCHL2 sequence (SEQ ID NO:20), and the *Citrus sinensis* chlorophyllase sequence (SEQ ID NO:22). The lipase and P-loop motifs discussed in Tsuchiya et al. (1999, *Proc. Natl. Acad. Sci. U.S.A.* 96:15362-15367) are underlined and the putative active Serine is written in white and boxed in black. Amino acids identical among all the sequences in the alignment are indicated by an asterisk (*) above the alignment. As can be seen in the figure, the amino acid sequence of SEQ ID NO:28 contains a motif containing Leu-Ala-Gly-His-Ser-Arg-Gly-Gly-Asp-Thr-Ala-Phe-Ala-Val-Ala-Leu which includes the putative active Serine.

The data in Table 6 presents the percent identity of the amino acid sequences set forth in SEQ ID NO:28, with the AtCHL1 sequence (NCBI General Identifier

No. 2460203, SEQ ID NO:21), the CaCHL sequence (NCBI General Identifier No. 6729675, SEQ ID NO:22), the AtCHL2 sequence (NCBI General Identifier No. 6729677, SEQ ID NO:20), and the *Citrus sinensis* chlorophyllase sequence (NCBI General Identifier No. 7328567, SEQ ID NO:22).

TABLE 6

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Chlorophyllase

Clone	SEQ ID NO.	Percent Identity to			
		2460203	6729677	7328567	6729675
csc1c.pk006.l9:fis	28	34.0	34.3	28.9	29.1

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS* 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

To determine if the insert in clone csc1c.pk006.l9:fis encodes an active chlorophyllase, the encoded polypeptide was expressed in *E. coli* and tested for chlorophyllase activity using essentially the method described in Example 7.

Initially, the chlorophyllase-coding sequence in clone csc1c.pk006.19 was amplified by PCR using *Pfu* polymerase (Stratagene) and the oligonucleotide primers P3 (sense, SEQ ID NO:29) and P4 (antisense, SEQ ID NO:30):

P3: 5'-TTCCATGGCGGCATCGCCGGTGGC-3' (SEQ ID NO:29)
P4: 5'-TTGCGGCCGCCAGCCTTGCTATTCAAGC-3' (SEQ ID NO:30)

The amplified DNA fragment was cloned into pCR-Script Amp SK(+) (Stratagene) according to the manufacturer's protocol. The resulting plasmid was digested with NcoI and NotI and the fragment corresponding to the chlorophyllase-cloning region of clone csc1c.pk006.l9:fis was purified and inserted into NcoI/NotI-cut *E. coli* expression vector pET24a (Novagen) to generate the plasmid pZmCHL.

Expression of the protein encoded by clone pZmCHL, its purification, and measurement of chlorophyllase activity will be as described in Example 7.

Sequence alignments, BLAST scores and probabilities, and chlorophyllase activity assays indicate that the nucleic acid fragment comprising the instant cDNA clone encodes one entire corn chlorophyllase isozyme of a class not previously known to Applicant.

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising:
 - (a) a nucleotide sequence encoding a polypeptide having chlorophyllase activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 26 have at least 80% sequence identity, or
 - (b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.
2. The polynucleotide of Claim 1 wherein the sequence identity is at least 90%.
3. The polynucleotide of Claim 1 wherein the sequence identity is at least 95%.
4. The polynucleotide of Claim 1 wherein the polynucleotide encodes the polypeptide sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 26.
5. The polynucleotide of claim 1 wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 25.
6. A vector comprising the polynucleotide of Claim 1.
7. A recombinant DNA construct comprising the polynucleotide of Claim 1 operably linked to at least one regulatory sequence.
8. A cell comprising the recombinant DNA construct of Claim 7.
9. The cell of Claim 8 wherein the cell is a plant cell or a bacterial cell.
10. A method for producing a plant comprising transforming a plant cell with the polynucleotide of Claim 1 and regenerating a plant from the transformed plant cell.
11. A plant comprising the recombinant DNA construct of Claim 7.
12. A seed comprising the recombinant DNA construct of Claim 7.
13. A method for transforming a cell, comprising introducing into a cell the polynucleotide of Claim 1.
14. A method for isolating a polypeptide encoded by the polynucleotide of Claim 1 comprising isolating the polypeptide from a cell transformed with said polynucleotide.

SEQ ID NO:19 MAAIEDSPTFSS--VVTCAA--FEIGSLPTEIPVDPV-----ENDST
SEQ ID NO:20 MS-SSSSR-----NAFEDGKYKSNLLTLD---SSSRCKITPSSRAS
SEQ ID NO:22 MAAMVDAKPAAS---VQGTPLLA---TATLPVFTRGISTKRITLETSSPS-----SP
SEQ ID NO:21 MAKLLLLIFGVFIFVNSQAQTFTILEKHNSEKITDVFHKGNFQVTNNPI-----RVKRYEF
SEQ ID NO: 2 -----RVET-----SNIA--
SEQ ID NO: 4 STCSTTAA-----NVFEIGKHITVLLRAE---PGT--C--TTKSSLPV
SEQ ID NO: 6 MNLASAVRVFLSYLLVQRMGS-----EQGGVFDQGGHVSLSLRLDEARAPRCVRSSPSSAAS
SEQ ID NO: 8 MAQRAEPILVT-----TDVFQMGNIQWKQFNIDT-----SNASS
SEQ ID NO:10 MAQRAQPVLAT-----DVFQMGNIQWKQFNVDV-----SSASF
SEQ ID NO:12 MAQRAQPALAT-----TDVFQKGDHVKQFNVDV-----STASS
SEQ ID NO:14 MASSPCS-----VFVPGKYTVQLKSVE---AGTK--KARHVSSVSA
SEQ ID NO:16 -----
SEQ ID NO:18 MAAAAPAEETMKNK---SAAGAEVP-----EAFTSVFQPGKLAVEAIQVDE-----NAAP
SEQ ID NO:26 MAAMA-----TTVFQAGPMEVDVKHVDK-----SMIP

FIG. 1A

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SEQ	ID NO: 19	APPKPV	RITCPTVAGTYPVVLEFFHGFYLRNYFYSDVLNHIASHGYILVAPQLCKLLPP--GGQ-VEVDDA
SEQ	ID NO: 20	SPPKQLLVATPVEEGDYPVVMLLHGYYLLYNFYSQMLHLVSSHGFILIAPOLYSIAGP--DTM-DEIKST	
SEQ	ID NO: 22	PPPKPLIIIVTPAGKGTENVILFLHGTSLSNKSYSKIFDHIASHGFIIVAPQLYTSIPPP-SAT-NELNSA	
SEQ	ID NO: 21	SAPEPLIIISPKEAGVPVLLFIHGTMLSNEDYSLFFNYIASHGFIIVAPKLFRFLFPKLPQQDEIDMA	
SEQ	ID NO: 2	SPPKPLLIVTPTTIQGTYPVLLFLHGFELRNTFTYTLQLLISSHGFIIVAPQLYGLLPP--SGI-QEIKSA	
SEQ	ID NO: 4	PPLQLLIA TPSEAGEEP LLLLHGYYLLYNFYSQLIQHIA SHGFI VLAPQLYTVAGP--DSS-EEIKSA	
SEQ	ID NO: 6	LPPKPLLVAAPRETGEYPVILFLHGYLA VNSFYSQLFEHVASHGFI VVG PQLYTISGA--DTT-EEINSA	
SEQ	ID NO: 8	SPPKPLLIFTPTVPGSYPVILFCHGFSLRNSYYSELLGHIA SHGFI I VAPQLCWSVRSMLEPG-DEVKEF.	
SEQ	ID NO: 10	SPPKPLLIFTPTVPGAYPVILFVHGF FIRNFYYSKLLAHIVSHGFI I VAPQLFSNGLPMYGP--TEVEYA	
SEQ	ID NO: 12	SPPKPLLIFTPTVPGLYPVILFCHGFCIRTSYYSKLLAHIVSHGFI I VAPQLFSIGVPMFGP--EEVKCE	
SEQ	ID NO: 14	PPRKPLLIATPFEEGEYPTLLLLHGEMLHNTFYSEL IQHIA SHGFI VVVPQLYL VATC--DST-NGIKSA	
SEQ	ID NO: 16	-----	
SEQ	ID NO: 18	TPPIPV LIVAPKDAGTYPVAMLLHGFE FLHNHFYEHL LRHVASHGFI I VAPQ-FSISI PSGDA-EDIAAA	
SEQ	ID NO: 26	NLARPLMVVAPKETGAYPVIVFLHGWNMLNSWYEQ LLTHVASHGFI AVAPQLYW MVSEP--DA-DDIDAT	

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FIG. 1B

SEQ ID NO:19	GSVINW-ASE--NLKAHLPT-----SVNANGKYTSLVGH	**	*	*	*	*	*	*	*	*
SEQ ID NO:20	AEIMDWLSV---GLNHFLPA-----QVTPNLSKFALS GH	**	*	*	*	*	*	*	*	*
SEQ ID NO:22	AEVAEWLPQ---GLQQLPE-----NTEANVSLVAVM GH	**	*	*	*	*	*	*	*	*
SEQ ID NO:21	ASVANWMPY---LQVVLQRYVT---GVEGDLEKLAI S GH	**	*	*	*	*	*	*	*	*
SEQ ID NO: 2	AAVTNWLS---GLQSVLPE-----NVKPDLLKLALS GH	**	*	*	*	*	*	*	*	*
SEQ ID NO: 4	AALTNWLSK---GLHDLPLP-----HVRPNLSKLG LAGH	**	*	*	*	*	*	*	*	*
SEQ ID NO: 6	AAVIDWLAT---GLPSTLPL-----GVRANLTKVSI S GH	**	*	*	*	*	*	*	*	*
SEQ ID NO: 8	GKVVDWLAEE---GLQPLLPE-----NVEAKLDKLVL S GH	**	*	*	*	*	*	*	*	*
SEQ ID NO:10	GKVADWI-AE---ELQHLLPE-----NVEANLDKLVL S GH	**	*	*	*	*	*	*	*	*
SEQ ID NO:12	GRVVDWL-DN---GLQPLLPE-----SVEAKLEKLV L VGH	**	*	*	*	*	*	*	*	*
SEQ ID NO:14	AKTTDWLKD---GLQDVLPT-----KVRPDLKKLGL S GH	**	*	*	*	*	*	*	*	*
SEQ ID NO:16	-----GLQDVLPT-----PRVEPDLSKLALAGH	**	*	*	*	*	*	*	*	*
SEQ ID NO:18	AKVADWLP-D---GLPSVLPK-----GVEPELSKLALAGH	**	*	*	*	*	*	*	*	*
SEQ ID NO:26	KRITNWLADHDKGLAHVLKDV LKLEHVEPDLSKLALAGH	**	*	*	*	*	*	*	*	*

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FIG. 1C

SEQ	ID	NO: 19	DPVAGTNKY--	IRTDPHILTYKPESEFELDI	PVAVVGTGLGP	KWNNVMPPCAP	TDLNHEEFYKE	--CKA
SEQ	ID	NO: 20	DPVDGTGKGK--	QT PPPV LAYLPNSFDL	DKTPILVIGSGLGETARNPL	FPPCAPPPGVNHR	EFFRE	--CQG
SEQ	ID	NO: 22	DPVAGTSKTTG--	LDPSILSF--	DSFDFSI	PVTVIGTGLGVAR--	CITACAP	EGANHEEFFNR--CKN
SEQ	ID	NO: 21	DPVAG--	RSVDDRTLPHVLTYPKPN	SENL SI	PVTVIGSGLGNHTIS	-----	CAPNHVSHQQFYDE--CKE
SEQ	ID	NO: 2	DPVGGLSKC--	CQTPVKILTYVPHSFNLAI	PVCVIGTGLGDEPRNCL	TCPCAPDGVNH	VEFFSE	--CKP
SEQ	ID	NO: 4	DPVDGMDKGK--	QT PPPV LTYVPHSFELDMA	VMVIGSGLGEVKNRPL	FPPCAPKGVNH	EDFFKE	--CRE
SEQ	ID	NO: 6	DPVDGMGVGK--	QT PPPILTGRHGS	LHVG-APAMVIGTGLGELPRGSL	LLPPCAPRGVSHAAFY	DEL	DGAA
SEQ	ID	NO: 8	DPVAGP--	CKSCETFPPI LTGMSQSENLNI	PIV VIGTGLGPEKANFFI	PPCAPDGVNHKE	FFNK	--CKP
SEQ	ID	NO: 10	DPVAGTSKY--	CRTRPHILTGKPRSF	DLKM-PVEVIGTGLGPEKLN	CCTPPCAPDGVN	YKEFFNE	--CKP
SEQ	ID	NO: 12	DPVAGVSKCKPCRSLP	DI LTGVPRSENLNI	PVAVIGTGLGPEKANSL	FPPCAPNGVN	HKEFFSE	--CKP
SEQ	ID	NO: 14	DPVDGVRKGH--	QTNPPVLNYI PHSLELKM	PSLVIGTGLGELKRN	-LFA-CAPKGVNH	QDFYDE	--CSS
SEQ	ID	NO: 16	DPVAGVSRAQ--	QLEPKVLT	FEPCLDVGM-PVLVMGTGLGPKHIGGF	--PCAPVGVNH	AAEFYKE	--CAP
SEQ	ID	NO: 18	DPVAGTGK--	SSQLQPKILTYEPSSFG	MAM-PVLVIGTGLGEEKKNI	FFPPCAPKDVNH	AAEFYRE	--CRP
SEQ	ID	NO: 26	DPVAGVSRAQ--	QLEPKVLT	FEPCLDVGM-PVLVMGTGLGPKHIGGF	--PCAPVGVNH	AAEFYKE	--CAP

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FIG. 1D

SEQ ID NO:19 TK-AHFVAADYGHMDMLDDDLPGFVG-FMAGCMCKNGQR-KKSEMRSFVGGIVVAFKYSYLWGEKAEIRL
SEQ ID NO:20 PA-WHEVAKDYGHLDMLDDDTKGIRG-KSSYCLCKNGEE--RRPMRRFVGGLVVSFLKAYLEGDDRELVK
SEQ ID NO:22 SSRAHFVATDYGHMDIILDDNPSDVKSWALSKYFCKNGNES-RDPMRRCSVSGIVVAFKDKDFYGDGEDFRQ
SEQ ID NO:21 NSS-HFVITKYGHMDMLNEFRLSPIAVTMSL-MCAQSF-RPKATMRRTLGGMVAFELNAYFRDDGRQYYA
SEQ ID NO: 2 PC-SHEVTTTEYGHLDMLDDHLSGCIGAI-SGYICKSGK-GPRDPMRRCSVGGLFVAFELKAYLEGQTDGDFKA
SEQ ID NO: 4 PA-CYFLAKDYGHLDMLDDETNNGIRG-KATHCLCKNGKS--REPMRRFVGGIVIAFMKAYLEGDNSSLIS
SEQ ID NO: 6 PA-CHLVVRDYGHTDMMDDDTPGARG-MLTRTICRSGGA--RAPMRRFVAGATVAFELKKVWVAGDAAAMDS
SEQ ID NO: 8 PC-AHEVATEYGHMDMLDDVTPGLIGSILSNICKDGK-GPRDLMRRTVGGLVVAFELRAQLNGLWKDFNA
SEQ ID NO:10 PC-AKFVVAKYGHMDMLNDDTPGLIGTLVSKCMCKNGTTPGPRDLMRRTVGGLVVVSFLRAQLNDLWKDFDA
SEQ ID NO:12 PS-AYFVATDYGHMDMLDDETPGVIGTMMSKCMCKNGKKGPRDLMRRTVGGLVVAFELRAQLNEQWKDFDA
SEQ ID NO:14 PS-CHEVAKDYGHMDMLDDHTDGPGRG-IVSYCMCRNGES--RTPMRLFVGGIVVAFELKACLQGDQDLRA
SEQ ID NO:16 PR-YHLVVKDYGHLDMLDDNVP----YIINNMCMRNQHDTKDLARRTMGGAMVAFELRAKLRI DVRLIA
SEQ ID NO:18 PC-YYFVTKDYGHLDMLDDDDAPKFI-----TCVCKDGN-GCKGKMRRCSVAGIMVAFELNAALGEKDADLEA
SEQ ID NO:26 PR-YHLVVKDYGHLDMLDDNVP----YIINNMCMRNQHDTKDLARRTMGGAMVAFELRAKLRI DVRLIA

350

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FIG. 1E

SEQ ID NO:19	IVKD-PSVSPAKLDPSPLEEASGI--F-----V
SEQ ID NO:20	IKDGCHEDVPVEIQEFVI-----M
SEQ ID NO:22	ILKD-PSFAPIKLDSEYIDASSMLTT-----THVKV
SEQ ID NO:21	I-----IANRSLAPTNLFAEKKGFNF-----ATTYA-----QL
SEQ ID NO: 2	IVDE-PDLAPVKLDPVEFIEA-----V
SEQ ID NO: 4	IRDG-HATAPVELQNVEFL-----V
SEQ ID NO: 6	ITAR-PDQAPVALSVVEF-----GDEKAIA
SEQ ID NO: 8	VLAN-PNLAPTKLDDVVVPA-----
SEQ ID NO:10	ILKD-PNLAPTEVDGVDYIPA-----
SEQ ID NO:12	ILAS-PNLAPAKLDDVRYLPT-----
SEQ ID NO:14	LKDN-PEISNINLSIVDFYDPDGLTKYFDGGSISTPHYAVQESHQQ
SEQ ID NO:16	IYHN-PEIAPAVLDQVDEFLP-----CFVGRPNPSS-----V
SEQ ID NO:18	ILRD-PAVAPTTLDPVEHRVA-----
SEQ ID NO:26	IYHN-PEIAPAVLDQVDEFLP-----CFVGRPNPSS-----V

351 397

FIG. 1F

SEQ ID NO:19	MAAI-----EDSPTFSSVTPAAFEIGSLP-----TTEIPVDPVENDSTAPPKPVITCP	*
SEQ ID NO:20	MSSS-----SSRNAFEDGKYKSNLLTLDSS-----SRCCKITPSSRASPSPPKQLLVATP	*
SEQ ID NO:22	MAAM-----VDAKPAASVQGTPLLATATLPVFTRGYISTKRITLETSSPSSPPPKPLIIVTP	
SEQ ID NO:21	MAKLLLLIFGVFIFVNSQAQTFPTILEKHNSEKIT-DVFHKGNFQVTNNPI-RVKRYEFSAPEPLIISP	
SEQ ID NO:28	MAAS-----P-----VAIGTAVFQRCPLRVEARHVD--YSQVPSVPKPLMVVAP	

1 70

SEQ ID NO:19	TVAGTYPVVLFFHGFYLRNYFYSDVLNHIASHGYILVAPQLCK-LLP--PGQVEVDDAGSVINWAS---	*
SEQ ID NO:20	VEEGDYPVVMLLHGYYLLYNSFYSQLMLHVSSHGFILIAQQLYS-IAG--PDTMDEIKSTAEIMDWLS---	
SEQ ID NO:22	AGKGTENVILFLHGTSLSNKSYSKIFDHIASHGFIVVAPQLYTSIPP--PSATNELNSAAEVAEWLP---	
SEQ ID NO:21	KEAGVYPVLLFIHGTMLSNEDYSLFFNYIASHGFIIVVAPKLFRLFPKLPQQDEIDMAASVANWMP---	
SEQ ID NO:28	TDAGVYPVAVFLHGCNTVNSWYESLLSHVASHGFIAVAPQLYCVTLN--MNDLKDIDATRQVTAWLADKQ	

71 140

SEQ ID NO:19	ENLKAHLPT-----SVNAN-GKYTSLVGH S RGGKTAFFAVALGHAATLDPS-----ITF	*
SEQ ID NO:20	VGLNHFLPA-----QVTPNLSKFA-LSGH S RGGKTAFFAVAL-KKFGYSSN-----LKI	
SEQ ID NO:22	QGLQONLPE-----NTEANVS-LVAVMGH S RGGQTAFALS-----R-----YGE	
SEQ ID NO:21	----LYLQVVLQRYVTGVEGDLEKL-AISGH S RGGKSAFALALGF-----SNIKLDVTF	
SEQ ID NO:28	QGLAHVLANILQ--LHGVVRPDL SRL-ALAGH S RGGDTAFFAVALGLGPAASDDDDNNADAGTSPAALPLKE	

121 180

FIG. 2A

```

** *** *      *      *      *      *      *      *      *
SEQ ID NO:19  SALIGIDPVAGTNKYIRTDPHILTYKPESFELD-IPVAVVGTGLGPK-WNNVMPPCAPTDLNHEEFYKEC
SEQ ID NO:20  STLIGIDPVDGTGKGKQTPPPVLAAYLPNSFDLDKTPILVIGSGLGETARNPLFPFCAPPVGNHREFFREC
SEQ ID NO:22  GAVIGLDPVAGTSKTTGLDPSILSF--DSFDFS-IPVTVIGTGLGGVARC--ITACAPEGANHEEFFNRC
SEQ ID NO:21  SALIGVDPVAGRSVDDRTLPHVLTYKPNSEFNL-IPVTVIGSGLGNHT-----ISCAPNHVSHQOQFYDEC
SEQ ID NO:28  SALIGVDPVAGLSKQAQVEPKVLTFRPRSLD-PGMPALVVGTGLGP--KHVGGPPCAPAGVNHAEFFYDEC
181 240
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* *      * *      * *      * *      * *      * *      * *
SEQ ID NO:19  KATKA-HFVAADYGHMDMLDDDLPGFVGF-MAGCMC-KNGQRKKSEMRSFVGGIVVAFKYSWLGEKAEI
SEQ ID NO:20  QGPAAW-HFVAKDYGHLDMLDDDDTKGIRGK-SSYCLC-KNGEERRP-MRRFVGGLVVSLKAYLEGDDREL
SEQ ID NO:22  KNSSRAHFVATDYGHMDIILDDNPSDVKSWALSKYFC-KNGNESRDPMRRCVSGIVVAFKDFEYFGDAEDF
SEQ ID NO:21  KENSS-HFVITKYGHMDMLNEFRLSPIAVTMSL-MCAQSFRP-KATMRRTLGIMVAFNLNAYFRDDGRQY
SEQ ID NO:28  -APPRYHVVLRDYGHMDMLDDDG---VPYVINNCMRNTKDTKLARRAIGGAVVAFLRATLEDDDEDL
241 300
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SEQ ID NO:19  RLIVKD-PSVSPAKLDPSELEEAS-----GIFV
SEQ ID NO:20  -VKIKD-GCHE----DVPVEIQE-F-----EVIM
SEQ ID NO:22  APIKLDSEVEYIDASSMLTTTHVKVRQILKD-PSF
SEQ ID NO:21  YAIIANR-SLAPTNL-FAEKKGFNGFATTYAQL
SEQ ID NO:28  KVVLENRPGLSPAVLDPVG-----HDLA
361 384
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FIG. 2B

SEQUENCE LISTING

<110> E.I. du Pont de Nemours and Company

<120> Chlorophyllases

<130> BB1477 PCT

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<150> 60/238,161

<151> 2000-10-05

<160> 30

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<213> Vitis sp.

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Val Thr Pro Thr Ile Gln Gly Thr Tyr Pro Val Leu Leu Phe Leu His
      20              25              30

Gly Phe Glu Leu Arg Asn Thr Phe Tyr Thr Gln Leu Leu Gln Leu Ile
      35              40              45

Ser Ser His Gly Phe Ile Val Val Ala Pro Gln Leu Tyr Gly Leu Leu
      50              55              60

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Pro Pro Ser Gly Ile Gln Glu Ile Lys Ser Ala Ala Ala Val Thr Asn
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 Ala Phe Ala Leu Ala Leu Gly Tyr Ala Asp Thr Ser Leu Asn Phe Ser
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 Ala Leu Leu Gly Leu Asp Pro Val Gly Gly Leu Ser Lys Cys Cys Gln
 130 135 140
 Thr Val Pro Lys Ile Leu Thr Tyr Val Pro His Ser Phe Asn Leu Ala
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 Cys Leu Thr Cys Pro Cys Ala Pro Asp Gly Val Asn His Val Glu Phe
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 Phe Ser Glu Cys Lys Pro Pro Cys Ser His Phe Val Thr Thr Glu Tyr
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 Gly His Leu Asp Met Leu Asp Asp His Leu Ser Gly Cys Ile Gly Ala
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 Ile Ser Gly Tyr Ile Cys Lys Ser Gly Lys Gly Pro Arg Asp Pro Met
 225 230 235 240
 Arg Arg Cys Val Gly Gly Leu Phe Val Ala Phe Leu Lys Ala Tyr Leu
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 275 280 285

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<213> Vitis sp.

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Ile Thr Val Leu Leu Arg Ala Glu Pro Gly Thr Cys Thr Thr Lys Ser
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Ser Leu Pro Val Pro Pro Pro Leu Gln Leu Leu Ile Ala Thr Pro Ser
      35              40              45

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Glu Ala Gly Glu Phe Pro Leu Leu Leu Leu Leu His Gly Tyr Leu Leu
      50              55              60

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Tyr Asn Ser Phe Tyr Ser Gln Leu Ile Gln His Ile Ala Ser His Gly
      65              70              75              80

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Phe Ile Val Leu Ala Pro Gln Leu Tyr Thr Val Ala Gly Pro Asp Ser
      85              90              95

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Ser Glu Glu Ile Lys Ser Ala Ala Ala Leu Thr Asn Trp Leu Ser Lys
      100              105              110

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Gly Leu His Asp Leu Leu Pro Pro His Val Arg Pro Asn Leu Ser Lys
      115              120              125

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Leu Gly Leu Ala Gly His Ser Arg Gly Gly Lys Thr Ala Phe Ala Leu
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Ala Leu Arg Lys Ala Ser Thr Ser Leu Lys Phe Ser Ala Leu Ile Gly
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Ile Asp Pro Val Asp Gly Met Asp Lys Gly Lys Gln Thr Pro Pro Pro
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Val Leu Thr Tyr Val Pro His Ser Phe Asp Leu Asp Met Ala Val Met
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Val Ile Gly Ser Gly Leu Gly Glu Val Lys Arg Asn Pro Leu Phe Pro
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Pro Cys Ala Pro Lys Gly Val Asn His Glu Asp Phe Phe Lys Glu Cys
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Arg Glu Pro Ala Cys Tyr Phe Leu Ala Lys Asp Tyr Gly His Leu Asp
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<210> 6

<211> 346

<212> PRT

<213> Zea mays

<400> 6

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20 25 30

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35 40 45

Pro Arg Cys Ala Val Arg Ser Ser Pro Ser Ser Ala Ala Ser Leu Pro
 50 55 60
 Pro Lys Pro Leu Leu Val Ala Ala Pro Arg Glu Thr Gly Glu Tyr Pro
 65 70 75 80
 Val Ile Leu Phe Leu His Gly Tyr Leu Ala Val Asn Ser Phe Tyr Ser
 85 90 95
 Gln Leu Phe Glu His Val Ala Ser His Gly Phe Ile Val Val Gly Pro
 100 105 110
 Gln Leu Tyr Thr Ile Ser Gly Ala Asp Thr Thr Glu Glu Ile Asn Ser
 115 120 125
 Ala Ala Ala Val Ile Asp Trp Leu Ala Thr Gly Leu Pro Ser Thr Leu
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 Pro Leu Gly Val Arg Ala Asn Leu Thr Lys Val Ser Ile Ser Gly His
 145 150 155 160
 Ser Arg Gly Gly Lys Val Ala Phe Ala Leu Ala Leu Gly His Ala Lys
 165 170 175
 Ala Lys Leu Ala Val Pro Leu Ala Ala Val Val Ala Val Asp Pro Val
 180 185 190
 Asp Gly Met Gly Val Gly Lys Gln Thr Pro Pro Pro Ile Leu Thr Gly
 195 200 205
 Arg His Gly Ser Leu His Val Gly Ala Pro Ala Met Val Ile Gly Thr
 210 215 220
 Gly Leu Gly Glu Leu Pro Arg Gly Ser Leu Leu Pro Pro Cys Ala Pro
 225 230 235 240
 Arg Gly Val Ser His Ala Ala Phe Tyr Asp Glu Leu Asp Gly Ala Ala
 245 250 255
 Pro Ala Cys His Leu Val Val Arg Asp Tyr Gly His Thr Asp Met Met
 260 265 270
 Asp Asp Asp Thr Pro Gly Ala Arg Gly Met Leu Thr Arg Thr Ile Cys
 275 280 285
 Arg Ser Gly Gly Ala Arg Ala Pro Met Arg Arg Phe Val Ala Gly Ala
 290 295 300
 Thr Val Ala Phe Leu Lys Lys Trp Val Ala Gly Asp Ala Ala Ala Met
 305 310 315 320
 Asp Ser Ile Thr Ala Arg Pro Asp Gln Ala Pro Val Ala Leu Ser Val
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 Val Glu Phe Gly Asp Glu Lys Ala Ile Ala
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<210> 7
 <211> 1174

<212> DNA

<213> Glycine max

<400> 7

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<210> 8

<211> 315

<212> PRT

<213> Glycine max

<400> 8

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Met Ala Gln Arg Ala Glu Pro Ile Leu Val Thr Thr Asp Val Phe Gln
 1             5             10             15

Met Gly Asn Ile Lys Trp Lys Gln Phe Asn Ile Asp Thr Ser Asn Ala
      20             25             30

Ser Ser Ser Pro Pro Lys Pro Leu Leu Ile Phe Thr Pro Thr Val Pro
      35             40             45

Gly Ser Tyr Pro Val Ile Leu Phe Cys His Gly Phe Ser Leu Arg Asn
      50             55             60

Ser Tyr Tyr Ser Glu Leu Leu Gly His Ile Ala Ser His Gly Phe Ile
      65             70             75             80

Ile Val Ala Pro Gln Leu Cys Trp Ser Val Arg Ser Met Leu Glu Pro
      85             90             95

Gly Asp Glu Val Lys Phe Ala Gly Lys Val Val Asp Trp Leu Ala Glu
      100            105            110

Glu Gly Leu Gln Pro Leu Leu Pro Glu Asn Val Glu Ala Lys Leu Asp
      115            120            125

Lys Leu Val Leu Ser Gly His Ser Lys Gly Gly Lys Thr Val Phe Ala
      130            135            140

Val Ala Leu Gly Tyr Ala Lys Thr Asn Leu Lys Phe Ser Ala Leu Val
      145            150            155            160

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Gly Ile Asp Pro Val Ala Gly Pro Cys Lys Ser Cys Glu Thr Phe Pro
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 Pro Ile Leu Thr Gly Met Ser Gln Ser Phe Asn Leu Asn Ile Pro Ile
 180 185 190
 Val Val Ile Gly Thr Gly Leu Gly Pro Glu Lys Ala Asn Phe Phe Ile
 195 200 205
 Pro Pro Cys Ala Pro Asp Gly Val Asn His Lys Glu Phe Phe Asn Lys
 210 215 220
 Cys Lys Pro Pro Cys Ala His Phe Val Ala Thr Glu Tyr Gly His Met
 225 230 235 240
 Asp Met Leu Asp Asp Val Thr Pro Gly Leu Ile Gly Ser Ile Leu Ser
 245 250 255
 Asn Cys Ile Cys Lys Asp Gly Lys Gly Pro Arg Asp Leu Met Arg Arg
 260 265 270
 Thr Val Gly Gly Leu Val Val Ala Phe Leu Arg Ala Gln Leu Asn Gly
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 290 295 300
 Thr Lys Leu Asp Asp Val Val Tyr Val Pro Ala
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<210> 9
 <211> 1104
 <212> DNA
 <213> Glycine max

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<210> 10
 <211> 313

<212> PRT

<213> Glycine max

<400> 10

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Phe Ser Pro Pro Lys Pro Leu Leu Ile Phe Thr Pro Thr Val Pro Gly
 35 40 45

Ala Tyr Pro Val Ile Leu Phe Val His Gly Phe Phe Ile Arg Asn Phe
 50 55 60

Tyr Tyr Ser Lys Leu Leu Ala His Ile Val Ser His Gly Phe Ile Ile
 65 70 75 80

Val Ala Pro Gln Leu Phe Ser Asn Gly Leu Pro Met Tyr Gly Pro Thr
 85 90 95

Glu Val Glu Tyr Ala Gly Lys Val Ala Asp Trp Ile Ala Glu Glu Leu
 100 105 110

Gln His Leu Leu Pro Glu Asn Val Glu Ala Asn Leu Asp Lys Leu Val
 115 120 125

Leu Ser Gly His Ser Arg Gly Gly Lys Thr Val Phe Ala Val Ala Leu
 130 135 140

Gly His Ala Lys Thr Asn Leu Lys Phe Ser Ala Leu Val Gly Ile Asp
 145 150 155 160

Pro Val Ala Gly Thr Ser Lys Tyr Cys Arg Thr Arg Pro His Ile Leu
 165 170 175

Thr Gly Lys Pro Arg Ser Phe Asp Leu Lys Met Pro Val Glu Val Ile
 180 185 190

Gly Thr Gly Leu Gly Pro Glu Lys Leu Asn Cys Cys Thr Pro Pro Cys
 195 200 205

Ala Pro Asp Gly Val Asn Tyr Lys Glu Phe Phe Asn Glu Cys Lys Pro
 210 215 220

Pro Cys Ala Lys Phe Val Val Ala Lys Tyr Gly His Met Asp Met Leu
 225 230 235 240

Asn Asp Asp Thr Pro Gly Leu Ile Gly Thr Leu Val Ser Lys Cys Met
 245 250 255

Cys Lys Asn Gly Thr Thr Gly Pro Arg Asp Leu Met Arg Arg Thr Thr
 260 265 270

Gly Gly Leu Val Val Ser Phe Leu Arg Ala Gln Leu Asn Asp Leu Trp
 275 280 285

Lys Asp Phe Asp Ala Ile Leu Lys Asp Pro Asn Leu Ala Pro Thr Glu
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Val Asp Gly Val Asp Tyr Ile Pro Ala
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<211> 1125
<212> DNA
<213> Glycine max

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aactggttct agtaggtcac agcaagggtg gaaaaacagc atttgctgtg gcacttggtt 480
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<211> 316
<212> PRT
<213> Glycine max

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Ser Ser Ser Pro Pro Lys Pro Leu Leu Ile Phe Thr Pro Thr Val Pro
35 40 45
Gly Leu Tyr Pro Val Ile Leu Phe Cys His Gly Phe Cys Ile Arg Thr
50 55 60
Ser Tyr Tyr Ser Lys Leu Leu Ala His Ile Val Ser His Gly Phe Ile
65 70 75 80
Leu Val Ala Pro Gln Leu Phe Ser Ile Gly Val Pro Met Phe Gly Pro
85 90 95
Glu Glu Val Lys Cys Glu Gly Arg Val Val Asp Trp Leu Asp Asn Gly
100 105 110
Leu Gln Pro Leu Leu Pro Glu Ser Val Glu Ala Lys Leu Glu Lys Leu
115 120 125

Val Leu Val Gly His Ser Lys Gly Gly Lys Thr Ala Phe Ala Val Ala
 130 135 140
 Leu Gly Tyr Cys Lys Thr Lys Leu Lys Phe Ser Ala Leu Ile Gly Ile
 145 150 155 160
 Asp Pro Val Ala Gly Val Ser Lys Cys Lys Pro Cys Arg Ser Leu Pro
 165 170 175
 Asp Ile Leu Thr Gly Val Pro Arg Ser Phe Asn Leu Asn Ile Pro Val
 180 185 190
 Ala Val Ile Gly Thr Gly Leu Gly Pro Glu Lys Ala Asn Ser Leu Phe
 195 200 205
 Pro Pro Cys Ala Pro Asn Gly Val Asn His Lys Glu Phe Phe Ser Glu
 210 215 220
 Cys Lys Pro Pro Ser Ala Tyr Phe Val Ala Thr Asp Tyr Gly His Met
 225 230 235 240
 Asp Met Leu Asp Asp Glu Thr Pro Gly Val Ile Gly Thr Met Met Ser
 245 250 255
 Lys Cys Met Cys Lys Asn Gly Lys Lys Gly Pro Arg Asp Leu Met Arg
 260 265 270
 Arg Thr Val Gly Gly Leu Val Val Ala Phe Leu Arg Ala Gln Leu Asn
 275 280 285
 Glu Gln Trp Lys Asp Phe Asp Ala Ile Leu Ala Ser Pro Asn Leu Ala
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 Pro Ala Lys Leu Asp Asp Val Arg Tyr Leu Pro Thr
 305 310 315

<210> 13

<211> 1444

<212> DNA

<213> Gesneriana

<400> 13

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<210> 14

<211> 336

<212> PRT

<213> Gesneriana

<400> 14

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Ser Val Ser Ala Pro Pro Arg Lys Pro Pro Leu Ile Ala Thr Pro Phe
35 40 45

Glu Glu Gly Glu Tyr Pro Thr Leu Leu Leu Leu His Gly Phe Met Leu
50 55 60

His Asn Thr Phe Tyr Ser Glu Leu Ile Gln His Ile Ala Ser His Gly
65 70 75 80

Phe Ile Val Val Val Pro Gln Leu Tyr Leu Val Ala Thr Cys Asp Ser
85 90 95

Thr Asn Gly Ile Lys Ser Ala Ala Lys Thr Thr Asp Trp Leu Lys Asp
100 105 110

Gly Leu Gln Asp Val Leu Pro Thr Lys Val Arg Pro Asp Leu Lys Lys
115 120 125

Leu Gly Leu Ser Gly His Ser Arg Gly Gly Lys Asp Ala Phe Ala Leu
130 135 140

Ala Leu Gly Tyr Ala Lys Thr Thr Leu Ser Phe Ser Ala Leu Ile Gly
145 150 155 160

Ile Asp Pro Val Asp Gly Val Arg Lys Gly His Gln Thr Asn Pro Pro
165 170 175

Val Leu Asn Tyr Ile Pro His Ser Leu Glu Leu Lys Met Pro Ser Leu
180 185 190

Val Ile Gly Thr Gly Leu Gly Glu Leu Lys Arg Asn Leu Phe Ala Cys
195 200 205

Ala Pro Lys Gly Val Asn His Gln Asp Phe Tyr Asp Glu Cys Ser Ser
210 215 220

Pro Ser Cys His Phe Val Ala Lys Asp Tyr Gly His Met Asp Met Leu
225 230 235 240

Asp Asp His Thr Asp Gly Pro Arg Gly Ile Val Ser Tyr Cys Met Cys
245 250 255

Arg Asn Gly Glu Ser Arg Thr Pro Met Arg Leu Phe Val Gly Gly Ile
260 265 270

Val Val Ala Phe Leu Lys Ala Cys Leu Gln Gly Asp Lys Gln Asp Leu
275 280 285

Arg Ala Leu Lys Asp Asn Pro Glu Ile Ser Asn Ile Asn Leu Ser Ile
290 295 300

Val Asp Phe Tyr Asp Pro Asp Gly Thr Leu Thr Lys Tyr Phe Asp Gly
305 310 315 320

Gly Ser Ile Ser Thr Pro His Tyr Ala Val Gln Glu Ser His Gln Gln
325 330 335

<210> 15

<211> 916

<212> DNA

<213> Triticum aestivum

<400> 15

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<211> 205

<212> PRT

<213> Triticum aestivum

<400> 16

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Lys Thr Lys Leu Glu Leu Lys Phe Ser Ala Leu Ile Gly Val Asp Pro
35 40 45

Val Ala Gly Val Ser Arg Ala Gln Gln Leu Glu Pro Lys Val Leu Thr
50 55 60

Phe Glu Pro Asp Cys Leu Asp Val Gly Met Pro Val Leu Val Met Gly
 65 70 75 80
 Thr Gly Leu Gly Pro Lys His Ile Gly Gly Phe Pro Cys Ala Pro Val
 85 90 95
 Gly Val Asn His Ala Glu Phe Tyr Lys Glu Cys Ala Pro Pro Arg Tyr
 100 105 110
 His Leu Val Val Lys Asp Tyr Gly His Leu Asp Met Leu Asp Asp Asn
 115 120 125
 Val Pro Tyr Ile Ile Asn Asn Cys Met Cys Met Arg Asn Gln His Asp
 130 135 140
 Thr Lys Asp Leu Ala Arg Arg Thr Met Gly Gly Ala Met Val Ala Phe
 145 150 155 160
 Leu Arg Ala Lys Leu Arg Ile Asp Val Arg Asp Leu Ile Ala Ile Tyr
 165 170 175
 His Asn Pro Glu Ile Ala Pro Ala Val Leu Asp Gln Val Asp Glu Phe
 180 185 190
 Leu Pro Cys Phe Val Gly Arg Pro Asn Pro Ser Ser Val
 195 200 205

<210> 17
 <211> 1242
 <212> DNA
 <213> Triticum aestivum

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<210> 18
 <211> 319
 <212> PRT
 <213> Triticum aestivum

<400> 18

Met Ala Ala Ala Ala Pro Ala Glu Thr Met Asn Lys Ser Ala Ala Gly
 1 5 10 15
 Ala Glu Val Pro Glu Ala Phe Thr Ser Val Phe Gln Pro Gly Lys Leu
 20 25 30
 Ala Val Glu Ala Ile Gln Val Asp Glu Asn Ala Ala Pro Thr Pro Pro
 35 40 45
 Ile Pro Val Leu Ile Val Ala Pro Lys Asp Ala Gly Thr Tyr Pro Val
 50 55 60
 Ala Met Leu Leu His Gly Phe Phe Leu His Asn His Phe Tyr Glu His
 65 70 75 80
 Leu Leu Arg His Val Ala Ser His Gly Phe Ile Ile Val Ala Pro Gln
 85 90 95
 Phe Ser Ile Ser Ile Ile Pro Ser Gly Asp Ala Glu Asp Ile Ala Ala
 100 105 110
 Ala Ala Lys Val Ala Asp Trp Leu Pro Asp Gly Leu Pro Ser Val Leu
 115 120 125
 Pro Lys Gly Val Glu Pro Glu Leu Ser Lys Leu Ala Leu Ala Gly His
 130 135 140
 Ser Arg Gly Gly His Thr Ala Phe Ser Leu Ala Leu Gly His Ala Lys
 145 150 155 160
 Thr Gln Leu Thr Phe Ser Ala Leu Ile Gly Leu Asp Pro Val Ala Gly
 165 170 175
 Thr Gly Lys Ser Ser Gln Leu Gln Pro Lys Ile Leu Thr Tyr Glu Pro
 180 185 190
 Ser Ser Phe Gly Met Ala Met Pro Val Leu Val Ile Gly Thr Gly Leu
 195 200 205
 Gly Glu Glu Lys Lys Asn Ile Phe Phe Pro Pro Cys Ala Pro Lys Asp
 210 215 220
 Val Asn His Ala Glu Phe Tyr Arg Glu Cys Arg Pro Pro Cys Tyr Tyr
 225 230 235 240
 Phe Val Thr Lys Asp Tyr Gly His Leu Asp Met Leu Asp Asp Asp Ala
 245 250 255
 Pro Lys Phe Ile Thr Cys Val Cys Lys Asp Gly Asn Gly Cys Lys Gly
 260 265 270
 Lys Met Arg Arg Cys Val Ala Gly Ile Met Val Ala Phe Leu Asn Ala
 275 280 285
 Ala Leu Gly Glu Lys Asp Ala Asp Leu Glu Ala Ile Leu Arg Asp Pro
 290 295 300
 Ala Val Ala Pro Thr Thr Leu Asp Pro Val Glu His Arg Val Ala
 305 310 315

<210> 19

<211> 324

<212> PRT

<213> Arabidopsis thaliana

<400> 19

Met Ala Ala Ile Glu Asp Ser Pro Thr Phe Ser Ser Val Val Thr Pro
 1 5 10 15

Ala Ala Phe Glu Ile Gly Ser Leu Pro Thr Thr Glu Ile Pro Val Asp
 20 25 30

Pro Val Glu Asn Asp Ser Thr Ala Pro Pro Lys Pro Val Arg Ile Thr
 35 40 45

Cys Pro Thr Val Ala Gly Thr Tyr Pro Val Val Leu Phe Phe His Gly
 50 55 60

Phe Tyr Leu Arg Asn Tyr Phe Tyr Ser Asp Val Leu Asn His Ile Ala
 65 70 75 80

Ser His Gly Tyr Ile Leu Val Ala Pro Gln Leu Cys Lys Leu Leu Pro
 85 90 95

Pro Gly Gly Gln Val Glu Val Asp Asp Ala Gly Ser Val Ile Asn Trp
 100 105 110

Ala Ser Glu Asn Leu Lys Ala His Leu Pro Thr Ser Val Asn Ala Asn
 115 120 125

Gly Lys Tyr Thr Ser Leu Val Gly His Ser Arg Gly Gly Lys Thr Ala
 130 135 140

Phe Ala Val Ala Leu Gly His Ala Ala Thr Leu Asp Pro Ser Ile Thr
 145 150 155 160

Phe Ser Ala Leu Ile Gly Ile Asp Pro Val Ala Gly Thr Asn Lys Tyr
 165 170 175

Ile Arg Thr Asp Pro His Ile Leu Thr Tyr Lys Pro Glu Ser Phe Glu
 180 185 190

Leu Asp Ile Pro Val Ala Val Val Gly Thr Gly Leu Gly Pro Lys Trp
 195 200 205

Asn Asn Val Met Pro Pro Cys Ala Pro Thr Asp Leu Asn His Glu Glu
 210 215 220

Phe Tyr Lys Glu Cys Lys Ala Thr Lys Ala His Phe Val Ala Ala Asp
 225 230 235 240

Tyr Gly His Met Asp Met Leu Asp Asp Asp Leu Pro Gly Phe Val Gly
 245 250 255

Phe Met Ala Gly Cys Met Cys Lys Asn Gly Gln Arg Lys Lys Ser Glu
 260 265 270

Met Arg Ser Phe Val Gly Gly Ile Val Val Ala Phe Leu Lys Tyr Ser
 275 280 285

Leu Trp Gly Glu Lys Ala Glu Ile Arg Leu Ile Val Lys Asp Pro Ser
290 295 300

Val Ser Pro Ala Lys Leu Asp Pro Ser Pro Glu Leu Glu Glu Ala Ser
305 310 315 320

Gly Ile Phe Val

<210> 20

<211> 318

<212> PRT

<213> Arabidopsis thaliana

<400> 20

Met Ser Ser Ser Ser Ser Arg Asn Ala Phe Glu Asp Gly Lys Tyr Lys
1 5 10 15

Ser Asn Leu Leu Thr Leu Asp Ser Ser Ser Arg Cys Cys Lys Ile Thr
20 25 30

Pro Ser Ser Arg Ala Ser Pro Ser Pro Pro Lys Gln Leu Leu Val Ala
35 40 45

Thr Pro Val Glu Glu Gly Asp Tyr Pro Val Val Met Leu Leu His Gly
50 55 60

Tyr Leu Leu Tyr Asn Ser Phe Tyr Ser Gln Leu Met Leu His Val Ser
65 70 75 80

Ser His Gly Phe Ile Leu Ile Ala Pro Gln Leu Tyr Ser Ile Ala Gly
85 90 95

Pro Asp Thr Met Asp Glu Ile Lys Ser Thr Ala Glu Ile Met Asp Trp
100 105 110

Leu Ser Val Gly Leu Asn His Phe Leu Pro Ala Gln Val Thr Pro Asn
115 120 125

Leu Ser Lys Phe Ala Leu Ser Gly His Ser Arg Gly Gly Lys Thr Ala
130 135 140

Phe Ala Val Ala Leu Lys Lys Phe Gly Tyr Ser Ser Asn Leu Lys Ile
145 150 155 160

Ser Thr Leu Ile Gly Ile Asp Pro Val Asp Gly Thr Gly Lys Gly Lys
165 170 175

Gln Thr Pro Pro Pro Val Leu Ala Tyr Leu Pro Asn Ser Phe Asp Leu
180 185 190

Asp Lys Thr Pro Ile Leu Val Ile Gly Ser Gly Leu Gly Glu Thr Ala
195 200 205

Arg Asn Pro Leu Phe Pro Pro Cys Ala Pro Pro Gly Val Asn His Arg
210 215 220

Glu Phe Phe Arg Glu Cys Gln Gly Pro Ala Trp His Phe Val Ala Lys
225 230 235 240

Asp Tyr Gly His Leu Asp Met Leu Asp Asp Asp Thr Lys Gly Ile Arg
 245 250 255
 Gly Lys Ser Ser Tyr Cys Leu Cys Lys Asn Gly Glu Glu Arg Arg Pro
 260 265 270
 Met Arg Arg Phe Val Gly Gly Leu Val Val Ser Phe Leu Lys Ala Tyr
 275 280 285
 Leu Glu Gly Asp Asp Arg Glu Leu Val Lys Ile Lys Asp Gly Cys His
 290 295 300
 Glu Asp Val Pro Val Glu Ile Gln Glu Phe Glu Val Ile Met
 305 310 315
 <210> 21
 <211> 347
 <212> PRT
 <213> Chenopodium album
 <400> 21
 Met Ala Lys Leu Leu Leu Leu Ile Phe Gly Val Phe Ile Phe Val Asn
 1 5 10 15
 Ser Gln Ala Gln Thr Phe Pro Thr Ile Leu Glu Lys His Asn Ser Glu
 20 25 30
 Lys Ile Thr Asp Val Phe His Lys Gly Asn Phe Gln Val Thr Asn Asn
 35 40 45
 Pro Ile Arg Val Lys Arg Tyr Glu Phe Ser Ala Pro Glu Pro Leu Ile
 50 55 60
 Ile Ile Ser Pro Lys Glu Ala Gly Val Tyr Pro Val Leu Leu Phe Ile
 65 70 75 80
 His Gly Thr Met Leu Ser Asn Glu Asp Tyr Ser Leu Phe Phe Asn Tyr
 85 90 95
 Ile Ala Ser His Gly Phe Ile Val Val Ala Pro Lys Leu Phe Arg Leu
 100 105 110
 Phe Pro Pro Lys Leu Pro Ser Gln Gln Asp Glu Ile Asp Met Ala Ala
 115 120 125
 Ser Val Ala Asn Trp Met Pro Leu Tyr Leu Gln Val Val Leu Gln Arg
 130 135 140
 Tyr Val Thr Gly Val Glu Gly Asp Leu Glu Lys Leu Ala Ile Ser Gly
 145 150 155 160
 His Ser Arg Gly Gly Lys Ser Ala Phe Ala Leu Ala Leu Gly Phe Ser
 165 170 175
 Asn Ile Lys Leu Asp Val Thr Phe Ser Ala Leu Ile Gly Val Asp Pro
 180 185 190
 Val Ala Gly Arg Ser Val Asp Asp Arg Thr Leu Pro His Val Leu Thr
 195 200 205

Tyr Lys Pro Asn Ser Phe Asn Leu Ser Ile Pro Val Thr Val Ile Gly
 210 215 220
 Ser Gly Leu Gly Asn His Thr Ile Ser Cys Ala Pro Asn His Val Ser
 225 230 235 240
 His Gln Gln Phe Tyr Asp Glu Cys Lys Glu Asn Ser Ser His Phe Val
 245 250 255
 Ile Thr Lys Tyr Gly His Met Asp Met Leu Asn Glu Phe Arg Leu Ser
 260 265 270
 Pro Ile Ala Val Thr Met Ser Leu Met Cys Ala Gln Ser Phe Arg Pro
 275 280 285
 Lys Ala Thr Met Arg Arg Thr Leu Gly Gly Ile Met Val Ala Phe Leu
 290 295 300
 Asn Ala Tyr Phe Arg Asp Asp Gly Arg Gln Tyr Tyr Ala Ile Ile Ala
 305 310 315 320
 Asn Arg Ser Leu Ala Pro Thr Asn Leu Phe Ala Glu Lys Lys Gly Phe
 325 330 335
 Asn Phe Gly Phe Ala Thr Thr Tyr Ala Gln Leu
 340 345

<210> 22
 <211> 329
 <212> PRT
 <213> Citrus sinensis

<400> 22
 Met Ala Ala Met Val Asp Ala Lys Pro Ala Ala Ser Val Gln Gly Thr
 1 5 10 15
 Pro Leu Leu Ala Thr Ala Thr Leu Pro Val Phe Thr Arg Gly Ile Tyr
 20 25 30
 Ser Thr Lys Arg Ile Thr Leu Glu Thr Ser Ser Pro Ser Ser Pro Pro
 35 40 45
 Pro Pro Lys Pro Leu Ile Ile Val Thr Pro Ala Gly Lys Gly Thr Phe
 50 55 60
 Asn Val Ile Leu Phe Leu His Gly Thr Ser Leu Ser Asn Lys Ser Tyr
 65 70 75 80
 Ser Lys Ile Phe Asp His Ile Ala Ser His Gly Phe Ile Val Val Ala
 85 90 95
 Pro Gln Leu Tyr Thr Ser Ile Pro Pro Pro Ser Ala Thr Asn Glu Leu
 100 105 110
 Asn Ser Ala Ala Glu Val Ala Glu Trp Leu Pro Gln Gly Leu Gln Gln
 115 120 125

Asn Leu Pro Glu Asn Thr Glu Ala Asn Val Ser Leu Val Ala Val Met
 130 135 140
 Gly His Ser Arg Gly Gly Gln Thr Ala Phe Ala Leu Ser Leu Arg Tyr
 145 150 155 160
 Gly Phe Gly Ala Val Ile Gly Leu Asp Pro Val Ala Gly Thr Ser Lys
 165 170 175
 Thr Thr Gly Leu Asp Pro Ser Ile Leu Ser Phe Asp Ser Phe Asp Phe
 180 185 190
 Ser Ile Pro Val Thr Val Ile Gly Thr Gly Leu Gly Gly Val Ala Arg
 195 200 205
 Cys Ile Thr Ala Cys Ala Pro Glu Gly Ala Asn His Glu Glu Phe Phe
 210 215 220
 Asn Arg Cys Lys Asn Ser Ser Arg Ala His Phe Val Ala Thr Asp Tyr
 225 230 235 240
 Gly His Met Asp Ile Leu Asp Asp Asn Pro Ser Asp Val Lys Ser Trp
 245 250 255
 Ala Leu Ser Lys Tyr Phe Cys Lys Asn Gly Asn Glu Ser Arg Asp Pro
 260 265 270
 Met Arg Arg Cys Val Ser Gly Ile Val Val Ala Phe Leu Lys Asp Phe
 275 280 285
 Phe Tyr Gly Asp Ala Glu Asp Phe Arg Gln Ile Leu Lys Asp Pro Ser
 290 295 300
 Phe Ala Pro Ile Lys Leu Asp Ser Val Glu Tyr Ile Asp Ala Ser Ser
 305 310 315 320
 Met Leu Thr Thr Thr His Val Lys Val
 325

<210> 23
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:PCR primer P1

<400> 23
 tttcatatgg cagcagcagc acccg

25

<210> 24
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:PCR primer P2

<400> 24

tttctcgagc tgcaatggta cttctgctc

29

<210> 25

<211> 1156

<212> DNA

<213> Triticum aestivum

<400> 25

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gcacgaggcc atctccgcgc tgcttccgct ctagcttcta gctagctaag taagatatgg 60
ctgcaatggc gacgacggtg ttccaggcgg ggccaatgga ggtggatgtg aagcacgtgg 120
acaagagtat gatcccgaac ctggccaggc cgttgatggt ggtggcgccc aaggagaccg 180
gcgcgtaccc cgtcatcgct ttccctgcacg gctggaacat gctcaacagc tggtagcagc 240
agctcctcac acacgtcgcc tcccatgggt tcatcgccgt cgcaccacag ctctactgga 300
tgggtgtccga gcccgatgcg gacgacatag acgccacaaa gcgaatcacc aactggcttg 360
cagatcatga caaggggctc gcccacgtcc tcaaggacgt gctcaaactt gagcatgtcg 420
agcctgacct gtccaagctg gccctagccg gccatagccg aggcggccag acggccttcg 480
ccgtcgccct gggactaggg gacgccaaga ccaagctgga gctcaagttc tccgccctca 540
tcggcgctcga ccccggtggc ggggtttcca gagcccaaca gttggagccc aaggtgtcga 600
cttttgaacc tgactgtctc gacgtgggga tgccggtgct ggtcatgggg actgggctgg 660
gtccaagca catcggcgga ttcccatgcy ccccggtggg cgtgaaccac gccgaattct 720
acaaggagtg cgcgcgcct cgtaccacc tcgtggtcaa ggactacggg catctcgaca 780
tgctggatga caatgtgccc tatatcatca acaactgcat gtgcatgagg aaccaacacg 840
acaccaaaga tcttgctagg aggaccatgg gaggagccat ggttgccctc ctcagggcta 900
aattgcgaat cgatgttcgt gatctcatcg ccatatatca taatcctgag atcgcgccag 960
ccgtcctgga ccaagttgat gagtttcttc cttgcttcgt tggacggcca aatccgtcgt 1020
ctgtgtgaga gttatattac gtgcctatgc ctatctatta ctctaaata aggtgtgttg 1080
gtgcatggcc ttgtcatggc tcatgactac tatagcacgt ccagtcttcc taattactaa 1140
aaaaaaaaa aaaaaa 1156

```

<210> 26

<211> 323

<212> PRT

<213> Triticum aestivum

<400> 26

```

Met Ala Ala Met Ala Thr Thr Val Phe Gln Ala Gly Pro Met Glu Val
 1              5              10              15

Asp Val Lys His Val Asp Lys Ser Met Ile Pro Asn Leu Ala Arg Pro
      20              25              30

Leu Met Val Val Ala Pro Lys Glu Thr Gly Ala Tyr Pro Val Ile Val
      35              40              45

Phe Leu His Gly Trp Asn Met Leu Asn Ser Trp Tyr Glu Gln Leu Leu
      50              55              60

Thr His Val Ala Ser His Gly Phe Ile Ala Val Ala Pro Gln Leu Tyr
      65              70              75              80

Trp Met Val Ser Glu Pro Asp Ala Asp Asp Ile Asp Ala Thr Lys Arg
      85              90              95

Ile Thr Asn Trp Leu Ala Asp His Asp Lys Gly Leu Ala His Val Leu
      100              105              110

```


Lys Asp Val Leu Lys Leu Glu His Val Glu Pro Asp Leu Ser Lys Leu
 115 120 125
 Ala Leu Ala Gly His Ser Arg Gly Gly Gln Thr Ala Phe Ala Val Ala
 130 135 140
 Leu Gly Leu Gly Asp Ala Lys Thr Lys Leu Glu Leu Lys Phe Ser Ala
 145 150 155 160
 Leu Ile Gly Val Asp Pro Val Ala Gly Val Ser Arg Ala Gln Gln Leu
 165 170 175
 Glu Pro Lys Val Leu Thr Phe Glu Pro Asp Cys Leu Asp Val Gly Met
 180 185 190
 Pro Val Leu Val Met Gly Thr Gly Leu Gly Pro Lys His Ile Gly Gly
 195 200 205
 Phe Pro Cys Ala Pro Val Gly Val Asn His Ala Glu Phe Tyr Lys Glu
 210 215 220
 Cys Ala Pro Pro Arg Tyr His Leu Val Val Lys Asp Tyr Gly His Leu
 225 230 235 240
 Asp Met Leu Asp Asp Asn Val Pro Tyr Ile Ile Asn Asn Cys Met Cys
 245 250 255
 Met Arg Asn Gln His Asp Thr Lys Asp Leu Ala Arg Arg Thr Met Gly
 260 265 270
 Gly Ala Met Val Ala Phe Leu Arg Ala Lys Leu Arg Ile Asp Val Arg
 275 280 285
 Asp Leu Ile Ala Ile Tyr His Asn Pro Glu Ile Ala Pro Ala Val Leu
 290 295 300
 Asp Gln Val Asp Glu Phe Leu Pro Cys Phe Val Gly Arg Pro Asn Pro
 305 310 315 320
 Ser Ser Val

<210> 27

<211> 1274

<212> DNA

<213> Zea mays

<400> 27

ccacgcgtcc gccgctactc acgccgtact gtctgatcgg caacacctcg tgtacacgta 60
 ggcccggaga tatggcggca tcgccggtgg ccatcggcac ggcggtgttc cagcgggggc 120
 cactccgcgt ggaggcgagg cacgtcgact acagccaggt cccagcgtg cccaagccgc 180
 tcatggtggt cgcgccacc gacgccggcg tctaccccggt ggcggtcttc ctgcacggct 240
 gcaacacggt caacagctgg tatgagagcc tcctgtcgca cgctcgcgtcc cacgggttca 300
 tcgccgtggc gcccagctc tactgctga cgctcaacat gaacgacttg aaggacatcg 360
 acgccaccag gcaggtcacc gcctggctcg ccgacaagca gcaaggcctg gcgcacgtgc 420
 tcgcccaacat cctccagctc cacggcgtga ggccggacct ctccaggctg gcgctggccg 480
 gccacagccg cggcggcgac acggccttcg ccgtggcact cgggctcgga cccgccgcct 540
 cggacgacga cgacaacaac gcagacgcag gcacatcgcc ggcggcggtg ccactcaagt 600
 tctccgcgct gatcggcgtg gaccccggtg cggggctatc caagcaggcg caggtggagc 660
 cgaagggtgct gaccttcggg ccccggtccc tcgaccggg gatgccggcg ctggtcgtcg 720

```

gcacggggct cggccccaag cacgtgggag ggccgcccgtg cgcccccgcg ggcgtcaacc 780
acgccgagtt ctacgacgag tgcgcgccgc cgcggtacca cgtcgtgctg agggactacg 840
ggcacatgga catgctggac gacgacggcg tgccctacgt catcaacaac tgcattgtgca 900
tgaggaacac caaggacacc aaggacctcg ccaggagggc catcggggga gccgtgggtgg 960
cgttcctcag ggccacgctg gaggacgacg acgaggatct caaggtcgtg ctcgagaacc 1020
gccctggcct ctcgccggcg gtgctggacc cagttgggca tgacttggct tgaatagcaa 1080
ggctggcgga gtgtgctaca ttgtcgctcc ttatttacac acacatacca catgtttctt 1140
ttccattttg tcctcgatcg tgtcatgtcc cacttgccac aactgtccag tccgctgggtg 1200
tccaatgaat agcaaggatg tgaccctaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1260
aaaaaaaaaa aaag 1274

```

<210> 28

<211> 333

<212> PRT

<213> Zea mays

<400> 28

```

Met Ala Ala Ser Pro Val Ala Ile Gly Thr Ala Val Phe Gln Arg Gly
  1             5             10             15

```

```

Pro Leu Arg Val Glu Ala Arg His Val Asp Tyr Ser Gln Val Pro Ser
          20             25             30

```

```

Val Pro Lys Pro Leu Met Val Val Ala Pro Thr Asp Ala Gly Val Tyr
          35             40             45

```

```

Pro Val Ala Val Phe Leu His Gly Cys Asn Thr Val Asn Ser Trp Tyr
          50             55             60

```

```

Glu Ser Leu Leu Ser His Val Ala Ser His Gly Phe Ile Ala Val Ala
          65             70             75             80

```

```

Pro Gln Leu Tyr Cys Val Thr Leu Asn Met Asn Asp Leu Lys Asp Ile
          85             90             95

```

```

Asp Ala Thr Arg Gln Val Thr Ala Trp Leu Ala Asp Lys Gln Gln Gly
          100            105            110

```

```

Leu Ala His Val Leu Ala Asn Ile Leu Gln Leu His Gly Val Arg Pro
          115            120            125

```

```

Asp Leu Ser Arg Leu Ala Leu Ala Gly His Ser Arg Gly Gly Asp Thr
          130            135            140

```

```

Ala Phe Ala Val Ala Leu Gly Leu Gly Pro Ala Ala Ser Asp Asp Asp
          145            150            155            160

```

```

Asp Asn Asn Ala Asp Ala Gly Thr Ser Pro Ala Ala Leu Pro Leu Lys
          165            170            175

```

```

Phe Ser Ala Leu Ile Gly Val Asp Pro Val Ala Gly Leu Ser Lys Gln
          180            185            190

```

```

Ala Gln Val Glu Pro Lys Val Leu Thr Phe Arg Pro Arg Ser Leu Asp
          195            200            205

```

```

Pro Gly Met Pro Ala Leu Val Val Gly Thr Gly Leu Gly Pro Lys His
          210            215            220

```

Val Gly Gly Pro Pro Cys Ala Pro Ala Gly Val Asn His Ala (Glu Phe
 225 230 235 240
 Tyr Asp Glu Cys Ala Pro Pro Arg Tyr His Val Val Leu Arg Asp Tyr
 245 250 255
 Gly His Met Asp Met Leu Asp Asp Asp Gly Val Pro Tyr Val Ile Asn
 260 265 270
 Asn Cys Met Cys Met Arg Asn Thr Lys Asp Thr Lys Asp Leu Ala Arg
 275 280 285
 Arg Ala Ile Gly Gly Ala Val Val Ala Phe Leu Arg Ala Thr Leu Glu
 290 295 300
 Asp Asp Asp Glu Asp Leu Lys Val Val Leu Glu Asn Arg Pro Gly Leu
 305 310 315 320
 Ser Pro Ala Val Leu Asp Pro Val Gly His Asp Leu Ala
 325 330

<210> 29

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR primer P3

<400> 29

ttccatggcg gcatcgccgg tggc

24

<210> 30

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR primer P4

<400> 30

ttgcggccgc cagccttgct attcaagc

28